Imaging of the endothelial dysfunction in coronary atherosclerosis

Jyrki T. Kuikka[1], Olli T. Raitakari[2], K. Lance Gould[3]

[1] Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital and Niuvanniemi Hospital, 70210 Kuopio, Finland
[2] Department of Clinical Physiology and the Turku PET Centre, University of Turku, Turku, Finland
[3] Weatherhead PET Center for Preventing and Reversing Atherosclerosis, University of Texas Medical School, Houston, Texas, USA

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Abstract. Coronary endothelial dysfunction is characterised by coronary vasoconstrictive responses to endothelium-dependent vasodilators. It is associated with coronary artery disease (CAD) and is considered an early phase of coronary atherosclerosis. Patients with CAD benefit from vigorous risk factor interventions and medical treatment, with a marked decrease in coronary events and an improvement in survival that are not reported following revascularisation procedures. Therefore, early detection of anatomical and functional changes in the coronary vasculature due to atherosclerosis provides the basis for integrated pharmacological, dietary and lifestyle modifications to prevent cardiovascular events and revascularisation procedures. The question arises as to whether these alterations in regional myocardial tone can be detected by any of the current non-invasive methods. Several methods are reviewed. We consider that intracoronary ultrasonography is the most accurate method, but non-invasive positron emission tomography and magnetic resonance imaging technology is of growing importance for identifying endothelial dysfunction of early coronary atherosclerosis.

Keywords: Atherosclerosis – Endothelium – Imaging – Myocardium – Vasodilation

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Introduction

Medical outcomes and resource utilisation may be optimised by early diagnosis of coronary artery disease (CAD) and stratification of cardiac risk as the basis for management. Physicians responsible for patients with CAD face complex choices among multiple tests and between vigorous medical treatment alone and revascularisation procedures, each of which has specific risks and benefits [1]. While coronary arteriography and revascularisation procedures are commonly used as the primary treatment for CAD, recent randomised trials have shown that with these procedures the risk of coronary events and cardiac death is not reduced [2, 3, 4, 5, 6, 7]. By contrast, vigorous medical and risk factor treatment markedly reduces coronary events and improves survival. However, with either medical treatment or revascularisation some risk of coronary events remains. The perceptual problem is that coronary events during vigorous medical treatment may be viewed as due to "not doing anything", a viewpoint favouring revascularisation procedures despite the aforementioned disappointing results.

Several reports have suggested that endothelial dysfunction occurs early in the development of coronary atherosclerosis [8, 9, 10, 11, 12, 13] and is associated with adverse outcomes not seen in subjects without endothelial dysfunction [9, 14]. Normally, endothelium plays a major role in thrombosis or thrombolysis, platelet and leucocyte interactions at the vessel wall, control of coronary blood flow and regulation of vascular tone and growth [14]. Endothelial dysfunction contributes to disease states by mediating coronary vasospasm, vasoconstriction, inflammation, leucocyte adhesion, thrombosis and/or abnormal proliferation of vascular endothelium, hypertension and atherosclerosis [8, 14, 15, 16, 17]. This early stage of atherosclerosis, marked by endothelial dysfunction, may last for years with a long silent phase prior to the onset of symptoms or clinical manifestations of occlusive disease. It is therefore important to identify early CAD manifesting as endothelial dysfunction in asymptomatic people in order to undertake vigorous preventive treatment in an individual at high risk.

This review addresses the benefits and limitations of several anatomical, functional and biochemical imaging

Jyrki T. Kuikka (✉)
Department of Clinical Physiology and Nuclear Medicine,
Kuopio University Hospital and Niuvanniemi Hospital,
70210 Kuopio, Finland
e-mail: jkuikka@uku.fi
Tel.: +358-17-173262, Fax: +358-17-173244

approaches for the assessment of endothelial dysfunction as a marker of early CAD.

Normal endothelial function

Vascular endothelium is normally a one-cell-thick lining of the coronary arterial wall. It responds to physical and chemical stimuli, such as changes in haemodynamic shear forces and blood-borne signals, by synthesising and releasing a variety of vasoactive regulators. Substances released by the endothelium include nitric oxide, endothelium-derived relaxing factor, prostacyclin, endothelins, endothelial cell growth factor(s), interleukins, adhesion molecules and fibrinolytic factors [14].

The endothelium has anticoagulant, antiplatelet and fibrinolytic functions. In addition, the normal endothelium plays an important role in vascular growth, leucocyte adhesion, immunological regulation, metabolism of circulating amines, lipoprotein metabolism and integration or transduction of blood-borne signals [18]. Through its vascular regulating functions, the endothelium also controls coronary arterial smooth muscle tone in response to pharmacological and physiologic stimuli [19].

An endothelium-derived relaxing factor (EDRF) was first suggested by Furchgott and Zawadzki [20]. The biological effects of EDRF are mediated by nitric oxide (NO), or by metabolite(s) closely related to NO. NO maintains baseline vasodilation that counteracts intrinsic arterial vasoconstrictor tone at rest in the coronary, systemic and pulmonary circulations. NO release is stimulated by increased flow and associated increased shear stress on the endothelium and by bradykinin, thrombin, acetylcholine, serotonin and a variety of other circulating endogenous or exogenous agents. These agents increase NO release by activating specific endothelial receptors in coronary arterial smooth muscle cells that reduce intracellular calcium concentration and lead to vasorelaxation.

Endothelial dysfunction

Experimental studies have shown that physical damage to the endothelium leads to formation of atherosclerotic plaque [9]. For example, experimental acute hypertension impairs endothelial integrity [21]. High LDL cholesterol, low HDL cholesterol, active or passive cigarette smoking, hypertension and diabetes are consistently associated with impaired endothelial physiology, even among otherwise healthy subjects without overt atherosclerosis [14]. A family history of premature CAD is associated with abnormal endothelial function even in young asymptomatic adults [22]. The various mechanisms whereby these risk factors cause endothelial damage are largely unknown; however, a common denominator for all these conditions is increased oxidative stress, which has therefore been suggested as an important cause of endothelial dysfunction.

Clinically, endothelial dysfunction may be manifest as (1) vasospasm, (2) thrombus formation, (3) hypertension and (4) atherosclerosis [23]. The exact mechanisms whereby risk factors cause endothelial dysfunction are largely unknown. However, after the atherosclerotic process has been initiated, several cellular processes, such as inflammation and lipoprotein oxidation within developing lesions, maintain endothelial damage and cause progressive disease.

Endothelial dysfunction promotes the formation of atherosclerotic lesions by several mechanisms, including increased monocyte adherence to the vascular wall, enhanced cell membrane permeability to monocytes, macrophages and lipoproteins which then accumulate in the vessel wall, increased platelet adherence, and increased smooth muscle cell migration and proliferation [18]. Decreased production and/or local bioavailability of NO is also characteristic of endothelial dysfunction. NO is a vasodilator, inhibits platelet adherence and aggregation, and suppresses smooth muscle proliferation and endothelial cell-leucocyte interactions; therefore, decreased NO activity contributes to the initiation and progression of atherosclerotic lesions [18]. Endothelial vasodilator function of the coronary microvessels is an important determinant of myocardial perfusion during periods of increased demand, and therefore microvascular endothelial dysfunction may play a particularly significant role in the pathogenesis of myocardial ischaemia [24].

In advanced or clinically manifest coronary atherosclerosis, endothelial dysfunction may reduce myocardial perfusion and cause myocardial ischaemia. Many reports have shown that cholesterol-lowering therapy decreases ischaemia [25, 26, 27, 28, 29, 30, 31, 32] and improves cardiovascular survival in patients with coronary atherosclerosis [33], despite only modest regression of stenoses or plaque size by coronary arteriography [34]. The substantial improvement in clinical symptoms and survival in the face of only small anatomical changes appears to be due to the beneficial effects of lipid lowering on arterial function, improved coronary vasodilation, plaque stabilisation and decreased risk of thrombosis, all of which are closely related to improved endothelial function.

Circulating markers of endothelial function

Endothelial injury releases various factors that can be detected in the circulation as markers of endothelial dysfunction, as listed in Table 1.

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NO synthesis [35, 36, 37, 38]. In subjects with hypercholesterolaemia, high levels of ADMA are associated with impaired endothelium-dependent vasodilation and reduced urinary nitrate excre-

Table 1. Circulating markers of endothelial function and adhesion molecules expressed on the surface of vascular endothelial cells (markers of increased permeability to leucocytes and inflammatory activation)

Circulating markers of endothelial function
Asymmetric dimethylarginine[a]
Endothelin-1[b]
von Willebrand factor[c]
Tissue plasminogen activator[d]
Plasminogen activator inhibitor[d]
Adhesion molecules
ICAM-1
VCAM-1
E-selectin
P-selectin

ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1

[a] Endogenous competitive inhibitor of NO synthase

[b] Endothelium-derived peptide with vasoconstrictor and mitogenic properties

[c] Glycoprotein synthesised by vascular endothelial cells (marker of "endothelial activation")

[d] Proteins released by endothelial cells; markers of excessive endothelial cell stimulation by thrombin and/or endothelial damage

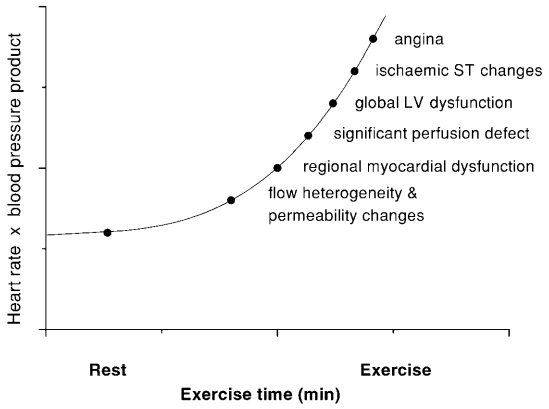

Fig. 1. Flow heterogeneity and permeability (endothelial) changes are most likely induced early during the course of exercise stress in patients with stable or unstable ischaemic syndromes, before the functional and metabolic abnormalities appear to produce ischaemic ST responses and anginal chest pain (modified from Fig. 3-15 of [98])

tion rates [39], suggesting that increased plasma levels of ADMA may be a circulating marker of endothelial dysfunction.

Subjects with coronary artery endothelial dysfunction have increased endothelin-1 immunoreactivity [40, 41]. Since injury to vascular endothelial cells triggers release of endothelin-1, circulating plasma endothelin levels may be a marker of injured dysfunctional endothelium.

Tissue plasminogen activator (TPA) and its primary inhibitor, plasminogen activator inhibitor-1 (PAI-1), maintain a physiological balance of fibrinolytic activity in the bloodstream. Both TPA and PAI-1 production are higher in atheromatous than in normal arteries [42, 43, 44], suggesting that increased TPA and PAI-1 levels may reflect excessive endothelial cell stimulation and/or endothelial damage, but they have poor specificity as markers of endothelial dysfunction.

von Willebrand factor is a glycoprotein synthesised by vascular endothelial cells that participates in the coagulation cascade and in the formation of platelet plugs at sites of endothelial damage [45, 46]. Increased levels of von Willebrand factor indicate endothelial activation or stimulation associated with increased secretion of the protein.

Binding of circulating leucocytes to the vascular endothelium and leucocyte migration into the subendothelial space are major processes in atherosclerosis [47, 48, 49, 50]. These events are mediated by a diverse family of cellular adhesion molecules that are expressed on the surface of vascular endothelial cells (Table 1). Circulating soluble forms of these adhesion molecules are elevated during inflammatory conditions and can be measured using commercial immunoassays.

Intramyocardial heterogeneity of coronary vasculature, flow and metabolism

Normal hearts demonstrate intramyocardial variation in coronary vasculature, flow, metabolism and endothelial function that blurs the definition of "normal" limits [51, 52, 53, 54, 55, 56]. Recognition and quantification of regional heterogeneity are important since changes in heterogeneity occur early in coronary atherosclerosis (Fig. 1). The pattern of heterogeneous myocardial perfusion as well as its change after stress or with time reflects the cumulative anatomical and functional status of the coronary vasculature and may serve both as a clinical marker of early coronary atherosclerosis and as the basis for early preventive treatment. Resting myocardial perfusion defects that improve after dipyridamole may be present in patients with arteriographically documented mild, non-obstructive CAD without flow-limiting defects [25].

When coronary perfusion pressure is reduced distal to significant coronary artery stenoses, the transmural perfusion is impaired, resulting in potential ischaemia with compensatory enhanced adenosine production and vasodilation more in the sub-endocardium than in the subepicardium [25, 53, 55]. With these changes in transmural perfusion heterogeneity, coronary arterial flow or average transmural absolute perfusion is less important for predicting ischaemia or severity of stenoses than the relative transmural distribution of perfusion, i.e. the intramyocardial perfusion heterogeneity. Similarly, exercise or pharmacological stress alters perfusion heterogeneity

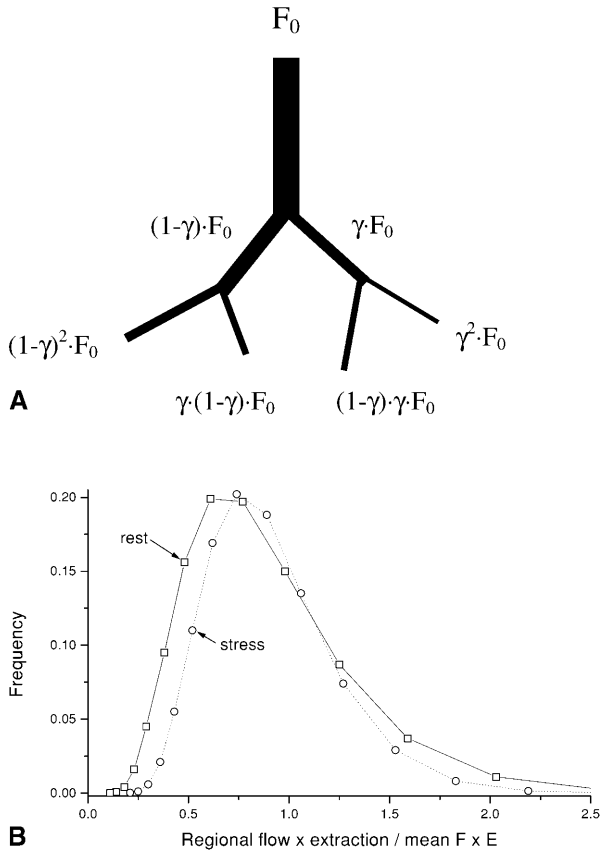

Fig. 2. A A simplified dichotomously branching myocardial flow model (modified from Fig. 10-8 of [52]) in two-dimensional space. Distribution of blood flow between daughter branches is characterised by an asymmetry parameter γ (γ<0.50). Regional flow after 2^n terminal branches is: $F_n=\gamma^k\cdot(1-\gamma)^{n-k}\cdot F_0$ and k=0,...,n. F_0 is the total blood flow into the coronary vasculature and n is the number of arterial branches. Gravity and physical-geometric properties of the vessels further complicate the model in three-dimensional space. **B** Frequency distribution of regional flows in a network with 15 arterial branches at rest and during adenosine stress. Regional flows were normalised to mean flow (=1.0). Distribution of regional flows was completely normal at rest (coefficient of variation =40%) but was more homogeneous during pharmacological stress (31%; data from sub-endocardial region of the anteroseptal wall in Fig. 3)

both transmurally and regionally, reflecting corresponding differences in intramyocardial microcirculatory vasomotion and metabolism.

Normally, intramyocardial perfusion varies over a tenfold flow range at rest and a fivefold range during hyperaemia [54, 55]. Within each layer of the normal myocardium, there is a high spatial variation of flow with time [56]. By contrast, in diseased segments, myocardial perfusion may become more homogeneous in affected regions owing to more uniform compensatory vasodilation. Similarly, in the well-perfused myocardium, local myocardial oxygen consumption varies more than threefold between low- and high-flow areas [53]. However, low-flow areas are not ischaemic since local lactate, adenosine and ATP are comparable to mean flow areas [53].

The key concepts here are heterogeneity and the integrated relations or effects of coronary vascular anatomy, function and perfusion. For example, quantitative analysis of the entire coronary arteriographic tree, including its anatomical heterogeneity (Fig. 2), predicts the normal coronary flow and flow reserve and on this basis the relative contribution of diffuse and segmental coronary artery narrowing to reduced myocardial perfusion and perfusion reserve in patients with CAD. This is reflected by new concepts in perfusion imaging [25, 57, 58].

From this perspective, let us now review current and/or novel imaging techniques for assessing coronary endothelial dysfunction associated with abnormal coronary vasculature, perfusion, perfusion reserve and metabolism.

Imaging techniques for assessing endothelial dysfunction

In vivo imaging of coronary endothelial dysfunction or its effects can be done with invasive or non-invasive techniques including quantitative coronary arteriography, intracoronary ultrasonography, intracoronary Doppler guidewire, venous occlusion plethysmography, functional magnetic resonance imaging, high-resolution ultrasonography, positron emission tomography and single-photon emission tomography (Table 2). Each technique has unique advantages and limitations. Any technique, however, will become limited as the threshold for identifying early coronary atherosclerosis is pushed to earlier and earlier stages. Even post-mortem histological examination, if performed at an early enough stage, may leave unresolved the question of whether atherosclerosis is present and, if so, whether it is in a form that will eventually become clinically manifest. Furthermore, endothelium may be dysfunctional as a result of either risk factors or heredity [25], and clinical CAD may be preventable by lowering risk factors to below a certain threshold.

Only very long-term follow-up of 10–30 years can elucidate what constitutes the forerunner of clinical disease. Moreover, the outcome is dependent on which interventions are made with respect to lifestyle and/or on which medications are given. Consequently, interpreting the importance of what we call endothelial dysfunction is complex and demands integrated reasoning, extrapolation and patient, very long-term observation inconsistent with the pace of research necessary for funding. In essence, the examination of earlier and earlier coronary atherosclerosis becomes limited by a biological uncertainty principle, i.e. as technology identifies mild atherosclerosis earlier, the prediction of or relation to clinical outcomes becomes more uncertain but at the same time more critical for establishing who needs lifelong treat-

Table 2. Invasive or non-invasive techniques for the in vivo imaging of coronary endothelial dysfunction or its effects

Invasive coronary arteriography[a]
Invasive forearm test – venous occlusion plethysmography[b]
Invasive intracoronary ultrasonography[c] and Doppler guidewire[d]
Non-invasive functional magnetic resonance imaging (fMRI)[e] and spectroscopy (MRS)
Non-invasive high-resolution ultrasonography[f]
Non-invasive positron (PET)[g] and single-photon emission tomography (SPET)[h]

[a] Coronary artery diameter is measured

[b] Changes in forearm flow are measured

[c] Changes in arterial diameter are measured. Currently, the most accurate invasive technique for identifying coronary atherosclerosis

[d] Coronary flow velocity and flow reserve are quantitatively measured. Pressure gradient can also be measured with a pressure guidewire

[e] Anatomy, function and perfusion are quantitatively measured. fMRI is a promising, high-resolution method for the assessment of myocardial anatomy, function and perfusion, but is work-in-progress. Measurements of cellular integrity with MRS are also far from clinical routine

[f] Peripheral arterial diameter is estimated

[g] Myocardial perfusion, perfusion reserve and metabolism are quantitatively measured. PET is today's gold standard for quantitative assessment of absolute perfusion and metabolism

[h] Function, perfusion, perfusion reserve and metabolism are estimated

ment and what form that treatment should take. In one sense, then, no technique will ever be completely adequate for detecting mild or diffuse coronary atherosclerosis because we do not know how to define it or precisely predict its outcome. However, current, more physiologically and spatially accurate techniques for the early diagnosis and management of coronary atherosclerosis have advanced to the point where these issues have to be considered.

Invasive coronary arteriography

Visually interpreted coronary arteriography is a poor standard for assessing coronary atherosclerosis and endothelial function. In vivo assessment of coronary endothelial function in humans was first reported by Ludmer and colleagues in 1986 [59]. The coronary artery diameter was measured by quantitative angiography, before and after intracoronary infusion of acetylcholine. In arteries with preserved endothelial function, acetylcholine stimulated the release of NO from endothelial cells, resulting in vasodilation, whereas in subjects with endothelial dysfunction, vasoconstriction was observed owing to the direct smooth muscle constrictor effect of acetylcholine in the absence of endothelial influence. These techniques have provided valuable insights into the risk factors for endothelial dysfunction in coronary arteries [60] and the role of endothelial dysfunction preceding overt atherosclerosis [9], and have also facilitated the prediction of long-term progression and cardiovascular events [61, 62].

In addition to its more common use to measure changes in arterial size after administration of endothelial pharmacological agents, advanced quantitative arteriography of the entire arteriographic tree directly identifies and quantifies early, diffuse coronary atherosclerosis. It utilises calibrated measurements of arteriographic luminal sizes, arterial lengths, branching patterns and localised stenoses throughout the coronary tree in comparison to the normally expected arterial dimensions for the size of each coronary arterial bed [25, 51, 62]. This technique can also be employed to calculate coronary flow reserve and flow heterogeneity due to diffuse coronary artery narrowing not apparent visually on the arteriogram based on integrated fluid dynamic analysis of the entire coronary artery tree. This quantitative analysis of the entire coronary tree has shown that diffuse 30%–35% narrowing along the length of a coronary artery that is not apparent visually decreases its calculated flow reserve to half or less of the normal value. In contrast, individual stenoses of 30%–35% that are visible on the arteriogram have little effect on flow reserve [25, 51].

Major disadvantages of coronary arteriography are its invasiveness, radiation exposure and the common use of visual interpretation of regional narrowing. Analysis of the entire coronary arteriographic tree requires specialised software, calibration of the cine system and substantial knowledge of the technique and fluid dynamics if accurate results are to be achieved.

Invasive venous occlusion plethysmography

Endothelial dysfunction can be considered a systematic disorder, which can also be identified in peripheral vasculature. Infusion of endothelial-dependent and -independent vasodilators in the brachial artery allows assessment of endothelial function in the forearm microcirculation by the measurement of changes in forearm flow using venous occlusion plethysmography techniques [63, 64]. Various drugs such as acetylcholine, methacholine, carbachol, bradykinin, serotonin and substance P have been used to assess endothelium-dependent vasodilation. Commonly used protocols involve infusion of acetylcholine with an arginine analogue such as L-N^G-monomethyl arginine (NMMA), which competitively inhibits NO synthesis. This combined pharmacological challenge allows the determination of the NO-dependent component of acetylcholine-stimulated flow. Disadvantages of venous occlusion plethysmography include its invasiveness and the fact that it measures peripheral microcirculation only.

Invasive intracoronary ultrasonography, Doppler guidewire and pressure guidewire

Intracoronary ultrasonography is the most accurate invasive technique for identifying coronary atherosclerosis [25]. The extent of atherosclerotic changes in angiographically normal or almost normal coronary arteries has been studied in patients with obstructive and non-obstructive CAD, high cholesterol levels [65], coronary vasospastic angina [66] and left ventricular hypertrophy [67]. In addition, intracoronary ultrasonography is used to follow changes in arterial diameter after intracoronary injection of endothelial active agents for testing of endothelial function [25].

Intracoronary Doppler guidewire provides direct quantitative measurements of coronary flow velocity and reserve [25, 68]. Absolute impairment of coronary flow velocity reserve correlates better with non-invasive functional measurements of stenosis severity than with arteriographic percent narrowing [69]. In humans, impaired coronary flow reserve is associated with endothelial dysfunction that improves with risk factor treatment. Intracoronary Doppler guidewire is also used to test endothelium-dependent vasodilation of resistance vessels in patients with CAD [70] or with idiopathic dilated cardiomyopathy [71].

More recently, the pressure guidewire has been used to assess diffuse coronary atherosclerosis in the absence of segmental narrowing by a distal to proximal pull back recording of the pressure gradient along the length of a coronary artery during adenosine-induced maximal coronary flow [25]. This graded pressure fall along the length of a diffusely narrowed coronary artery measured by intracoronary pressure wire corresponds to the longitudinal, base to apex perfusion gradient observed by positron emission tomography due to diffuse coronary atherosclerosis in the absence of flow-limiting stenoses [58]. Both techniques identify early diffuse CAD not apparent on coronary arteriograms as segmental stenoses.

The main drawbacks of these intracoronary techniques are their invasiveness, their uncommon use in clinical practice and the limited understanding that many investigators have of the complex underlying fluid dynamics.

Functional magnetic resonance imaging

Functional magnetic resonance imaging (fMRI) is a high-resolution non-invasive technique that does not require radionuclides or X-ray contrast agents for assessment of regional myocardial perfusion and perfusion reserve. In order to assess myocardial perfusion, this technique uses an inversion recovery, snapshot fast, low angle, short (FLASH) fMRI sequence, where a bolus flow of the contrast agent (gadopentetate dimeglumine; Gd-DTPA) is tracked through the myocardium (Fig. 3) [72,

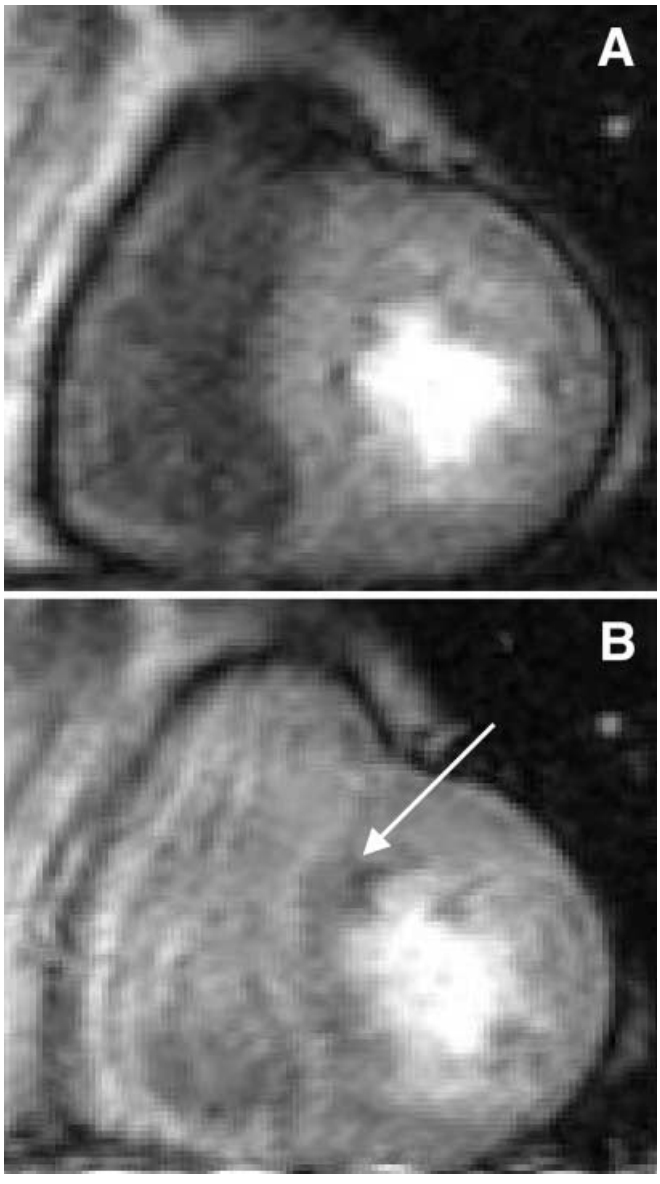

Fig. 3A, B. Hypertrophic cardiomyopathy is associated with both attenuated endothelial and non-endothelial coronary perfusion reserve. The first-pass cardiac MRI demonstrates normal myocardial perfusion (0.8 ml min^{-1} g^{-1}) at rest (**A**) and decreased perfusion (1.7 ml min^{-1} g^{-1}) during adenosine (a smooth muscle vasodilator and modulator of cardiac function) stress (**B**) after bolus injection of polylysine Gd-DTPA in a 50-year-old male subject with suspected hypertrophic cardiomyopathy [99] but angiographically normal coronaries. The stress scan was performed 30 min after resting injection. The *white arrow* (**B**) shows decreased perfusion in the sub-endocardial region of the anteroseptal wall. Anteroseptal ejection fraction, wall motion and tissue thickening were also abnormal. The results suggest the mechanism discovered by Depre et al. [100], namely, the decrease in cardiac workload induces similar patterns of gene response. This study was performed with the co-operation of Drs. Petri Sipola, Johanna Kuusisto, Keijo Peuhkurinen et al. at the Departments of Clinical Radiology and Cardiology, Kuopio University Hospital

73, 74]. Methodologically, it is similar to a combined first-pass and myocardial perfusion radionuclide study. The first-transit, time-intensity curves of Gd-DTPA for blood pool (left ventricular lumen or ascending/descending aorta) and for myocardial regions are measured, then the blood and myocardial signal intensities are converted to Gd-DTPA concentrations according to an in vitro calibration curve [73]. The unidirectional transfer rate constant for the uptake of Gd-DTPA into myocardium is computed, based on Kety's formula [73], as the product of perfusion and extraction of Gd-DTPA across the capillary membrane in ml min^{-1} g^{-1}. The extraction is assumed to be a constant, which is not true in reality. Increased flow decreases extraction.

High-resolution MRI potentially allows simultaneous measurements of percent diameter stenoses in addition to myocardial perfusion [75, 76, 77], with an edge-detection algorithm to objectively determine relative severity. Results of MRI studies have demonstrated that regions supplied by coronary arteries with <40% diameter stenoses have a significantly lower perfusion reserve than is present in healthy volunteers [74].

Limitations of fMRI are its restricted availability, the number of assumptions of the perfusion model, errors in calculation of the true arterial input function and the use of breath-holding to suppress respiratory motion, which limits the maximal duration of scanning and/or precludes use of fMRI in some patients. The "black-blood" technique and other fast MRI applications may provide direct plaque imaging as well as coronary arteriograms [75, 76, 77]. However, even using high-quality arteriograms, correct edge detection to the resolution required is difficult; there is a consequent substantial variation in the reliability of quantitative coronary arteriography in addition to the limitations of percent diameter narrowing as a measure of severity. These limitations also apply to MRI techniques that remain to be validated for clinical use.

Non-invasive high-resolution ultrasonography

Endothelial function may be studied non-invasively in the peripheral conduit arteries, using high-resolution external vascular ultrasound. In this method, arterial diameter is measured in response to an increase in shear stress, which causes endothelium-dependent dilatation, and in response to sublingual nitroglycerin, an endothelium-independent dilator. The brachial arterial dilator response to shear stress has been shown to be due mainly to endothelial release of NO [78].

Being non-invasive, the ultrasound method has been applied widely to asymptomatic subject groups, including children and young adults. By using this test, endothelial dysfunction has been demonstrated in asymptomatic children and young adults with risk factors for atherosclerosis, such as hypercholesterolaemia and cigarette smoking [79].

Disadvantages of high-resolution ultrasonography are the technical difficulty of obtaining reliable measurements, which requires a skilled sonographer, and methodological errors within individuals. The results of brachial artery ultrasonography have been reported to correlate with invasive testing of coronary endothelial function [80] and with the extent or severity of coronary atherosclerosis [81]. Although it is possible that endothelial function measured at one site of the arterial tree reflects the situation at other sites, this hypothesis needs further testing. Consequently, the major problem in the brachial artery test remains extrapolation of the results to the coronary circulation.

Positron emission tomography

Positron emission tomography (PET) permits non-invasive quantitation of relative or absolute myocardial perfusion by measuring blood flow at rest and after dipyridamole or adenosine, coronary blood flow and flow reserve (defined as the ratio of maximal flow to basal flow). Recent studies have shown that myocardial perfusion reserve measured using oxygen-15 labelled water or nitrogen-13 labelled ammonia is reduced in non-stenotic arteries of patients with arteriographic disease in other coronary arteries, in asymptomatic subjects with hypercholesterolaemia, in patients with diabetes and in patients with borderline hypertension [12, 82, 83, 84, 85, 86, 87]. These findings suggest that this impairment in coronary reactivity is an early marker of subclinical coronary atherosclerosis associated with endothelial dysfunction.

The exact defect in myocardial vasodilatory function responsible for the blunted response after dipyridamole is unknown, but the latter may be due to dysfunction of endothelial cells and/or smooth muscle cells of the macro- and/or the microcirculation. In addition to its direct smooth muscle effect, the increased shear stress caused by increased flow after intravenous dipyridamole may normally release vasodilating substances from the endothelium that elicit more prominent vasodilation with preserved endothelial function [88, 89] but impaired vasodilation with endothelial dysfunction. The myocardial flow response to dipyridamole or adenosine may therefore be regarded as an integrated measure of coronary reactivity including vascular smooth muscle relaxation and endothelial function, both of which may become disturbed in the early stages of atherosclerosis. In addition, PET is useful for following responses to lifestyle modification and pharmacological treatment of atherosclerosis [12, 26, 28, 30, 31]. More recently, it has been suggested that resting perfusion defects on PET (Fig. 4) which improve after dipyridamole may be markers of endothelial dysfunction before flow-limiting stenoses are present [25, 31].

The major disadvantages of PET are its high cost, its limited availability and radiation exposure.

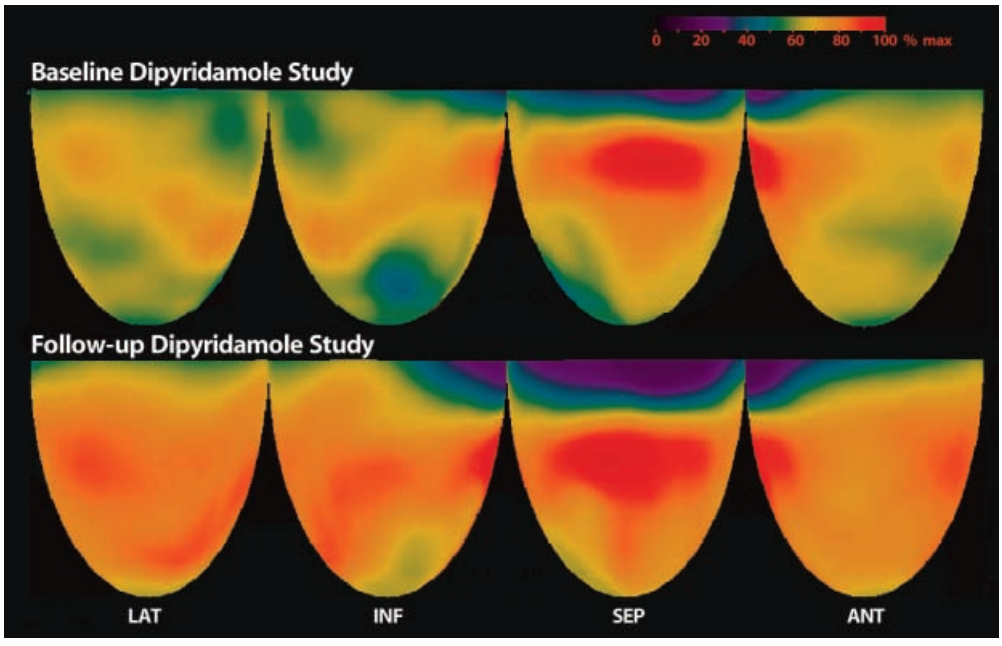

Fig. 4. Improved myocardial perfusion revealed by dipyridamole PET after 10 months of intense lifestyle and pharmacological treatment. The baseline study shows diffuse disease with a base to apex, longitudinal perfusion gradient after dipyridamole, as previously described [58], in addition to regional perfusion defects. After intense treatment, these abnormalities substantially resolved, suggesting soft resorbable plaques and functional restriction of coronary flow reserve at baseline rather than fixed structural stenoses

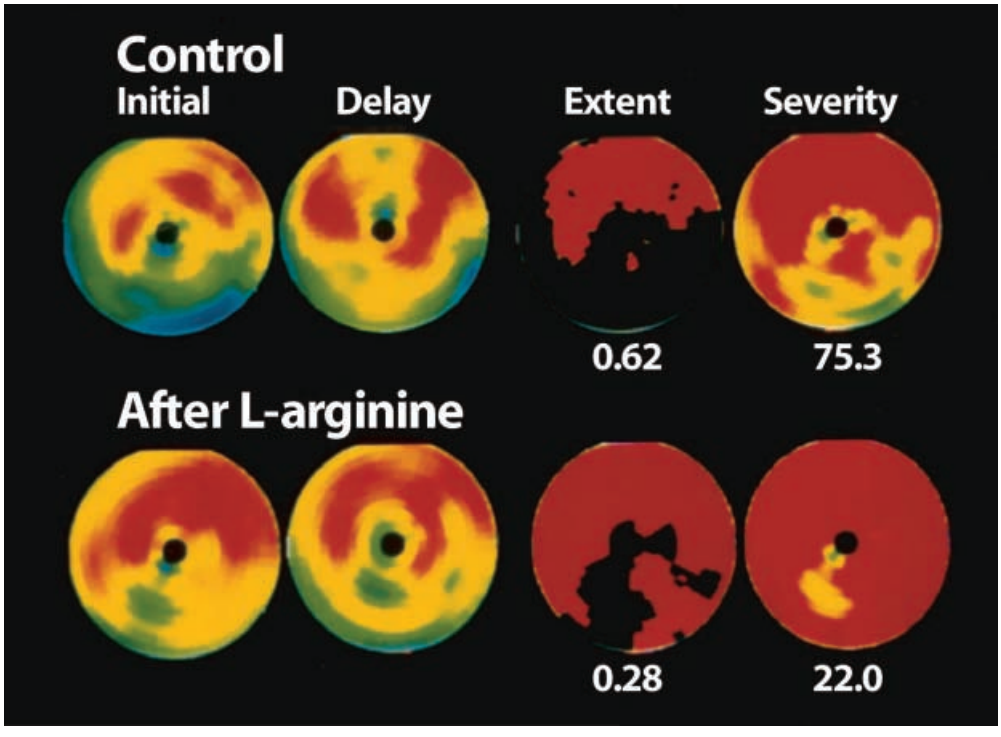

Fig. 5. Representative patient who demonstrated disappearance of redistribution and improvement of thallium perfusion abnormality on L-arginine test [93] (with permission of the American Society of Nuclear Cardiology)

Single-photon emission tomography (SPET)

Single-photon emission tomography (SPET) techniques with thallium-201 and technetium-99m labelled tracers are used to assess relative perfusion reserve by comparing the regional perfusion of ischaemic regions with the presumably normally perfused reference regions. Because coronary atherosclerosis and endothelial dysfunction may be macroscopically diffuse, assessment of the relative perfusion reserve by SPET imaging may not identify early disease [90, 91], normal images being obtained despite reduced absolute perfusion or perfusion reserve. On the other hand, SPET has shown improvement in the perfusion defects of patients with flow-limiting stenoses undergoing risk factor treatment [27, 31, 32].

Wieneke et al. [92] compared patients with angiographically unobstructed coronary arteries with and without stress perfusion defects using ^{201}Tl. The patients with abnormal perfusion images showed a significantly

lower increase in coronary flow on intracoronary Doppler after intracoronary injection of the endothelial-dependent vasodilator acetylcholine. Perfusion defects on radionuclide imaging occurred only if coronary flow velocity reserve as measured by intracoronary Doppler was significantly reduced in the corresponding perfusion area.

In the study by Fujita et al. [93], patients with angina pectoris and normal coronary arteries underwent exercise ^{201}Tl imaging without medication (baseline) and after intravenous administration of L-arginine (2.5 mmol/kg for 30 min) (Fig. 5). In 7 of the 12 patients whose ^{201}Tl redistribution disappeared after L-arginine, the percent increase in serum L-citrulline concentration during exercise was significantly larger than that in the remaining five patients (18% vs 0.9%, $P<0.01$). The epicardial coronary vasoconstriction in response to acetylcholine was also greater in the first group (28% vs 11%, $P<0.05$). Exogenous L-arginine improved myocardial perfusion during exercise, probably via increasing production of nitric oxide.

The main shortcomings of myocardial perfusion SPET are relatively poor spatial resolution, low count statistics and attenuation artefacts that preclude both identification of mild to moderate perfusion abnormalities of early CAD and assessment of absolute perfusion and perfusion reserve.

Clinical implications

Endothelial dysfunction is considered an early phase of coronary atherosclerosis, or as due to risk factors associated with CAD. While there are few studies on the outcomes in patients with endothelial dysfunction due to mild or diffuse CAD without flow-limiting stenoses, their results are suggestive. Suwaidi et al. [94] followed for 28 months 157 patients with CAD who were divided into three groups on the basis of their response to acetylcholine: group 1 (n=83), patients with normal endothelial function; group 2 (n=32), patients with mild endothelial dysfunction; and group 3 (n=42), patients with severe endothelial dysfunction. During follow-up, none of the patients from groups 1 and 2 had cardiac events, whereas six patients (14%) with severe endothelial dysfunction had ten cardiac events, including myocardial infarction, coronary revascularisation and cardiac death.

Schachinger and co-workers [15] assessed coronary vasoreactivity in 147 patients with non-obstructive CAD over a median follow-up period of 7.7 years. They also found that impaired endothelial and endothelium-independent coronary vasoreactivity was associated with a significantly higher incidence of cardiovascular events. The results of these studies support the hypothesis that endothelial dysfunction plays a role in the progression of coronary atherosclerosis and clinical events.

Consequently, it is important to develop further non-invasive methods for (a) identification of patients with endothelial dysfunction or mild or diffuse CAD and (b) assessment of the response to aggressive risk factor treatment by lifestyle modification and the use of cholesterol-lowering agents [57, 94]. "Absolute" quantitation of regional perfusion changes and more sophisticated analyses of relative perfusion with PET [58], SPET or fMRI are promising. Development of specific tracers for targeting atherosclerotic lesions and for specific endothelial cell membrane receptors [95, 96] and the use of magnetic resonance spectroscopy [97] to measure endothelial function and cellular integrity are interesting avenues for further research, but their clinical application is currently remote. Preliminary reports suggest that non-invasive fMRI, PET and intracoronary techniques are of growing importance for the identification of endothelial dysfunction as an early marker of coronary atherosclerosis and a basis for risk factor treatment.

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