Measurement of coronary vasomotor function: getting to the heart of the matter in cardiovascular research

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ABSTRACT

Measurement of endothelial function in patients has emerged as a useful tool for cardiovascular research. Although no gold standard for the measurement of endothelial function exists, the measurement of flow-mediated dilation in the brachial artery, assessed with Doppler ultrasonography, is the most studied method. However, the assumption that endothelial dysfunction detected in brachial arteries is a manifestation of systemic endothelial dysfunction including the coronary circulation may not be entirely valid. Brachial and myocardial circulations differ in terms of the microvascular architecture, the pattern of blood flow and vascular resistance (e.g. shunt vessels occur in the hand but not in the myocardium), their metabolic regulation, type of receptors that contribute to humoral regulation and the pathways that are activated to induce hyperaemia. In this context, measuring coronary vasomotor function may be more useful than brachial artery measures to predict and assess potential myocardial damage related to limited vascular responsiveness. This review aims to provide an overview of the basic concept of coronary flow reserve and its different modalities of measurement, as well as its utility in cardiovascular research.

INTRODUCTION

Measurement of endothelial function in patients has emerged as a useful tool for cardiovascular research. In the presence of atherosclerotic risk factors, the endothelium loses its normal regulatory functions [1]. Clinical syndromes such as stable and unstable angina, acute myocardial infarction, claudication and stroke may relate to a loss of endothelial control of vascular tone, thrombosis and the composition of the vascular wall. Recent studies have shown that the severity of endothelial dysfunction relates to the risk for an initial or recurrent cardiovascular event [2]. Finally, a growing number of interventions known to reduce cardiovascular risk also improve endothelial function [3]. This work has prompted speculation that endothelial function serves as a ‘barometer’ for cardiovascular health that can be used for patient care and evaluation of new therapeutic strategies [2,3]. The measurement of FMD (flow-mediated dilation) in the brachial artery, assessed with Doppler ultrasonography, is the most frequently used method [4]. It is a well-tolerated, non-invasive and low-risk procedure. Brachial artery FMD after transient vascular occlusion may serve as an index of NO (nitric oxide) bioavailability, and its impairment correlates with coronary arterial abnormalities [4]. These factors, with the

Key words: coronary microcirculation, coronary flow reserve, echocardiography, endothelial dysfunction, vasomotor function.

Abbreviations: CVR, coronary velocity reserve; FMD, flow-mediated dilation; FFR, fractional flow reserve; LAD, left anterior descending; LDL, low-density lipoprotein; LV, left ventricular; MDV, mean diastolic velocities; MR, magnetic resonance; NO, nitric oxide; PET, positron emission tomography; TEE, transoesophageal echocardiography.

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CORONARY FLOW OR BRACHIAL ARTERY FLOW TO ASSESS ENDOTHELIAL DYSFUNCTION

However, the assumption that endothelial dysfunction detected in brachial arteries is a manifestation of systemic endothelial dysfunction including the coronary circulation may not be entirely valid. Brachial and myocardial circulations differ in terms of the microvascular architecture, the pattern of blood flow and vascular resistance (e.g. shunt vessels occur in the hand but not in the myocardium), their metabolic regulation, type of receptors that contribute to humoral regulation and the pathways that are activated to induce hyperaemia [5,6]. Indeed, endothelial dysfunction seen in cardiovascular disease may have a more profound impact on the heart than on skeletal muscle both because the heart is uniquely vulnerable to ischaemia and because coronary circulation depends on two major principles that are unique to the heart [5,6]. The first is autoregulation, which is highly dependent on functional endothelium and ensures a tight link between blood supply and metabolic demand [7,8]. The second is the existence of the ‘zero pressure flow phenomenon’, whereby the coronary microcirculation may cease if the perfusion drops below a threshold level [9]. Endothelial-mediated vasodilation is probably an important regulator mechanism to lower resistance pressure and prevent no-flow.

Not only does endothelial dysfunction have a significant consequence in the heart, but also the impact of endothelium-independent vasoregulation appears to be additionally relevant in the heart. Coronary flow can reach five times that of resting flow during reactive hyperaemia to meet the high metabolic demand [6,10]. If myocardial damage is more likely to occur during periods of high metabolic demands as hypothesized by Vatner and Hittinger [11], endothelial dysfunction may not completely characterize the vascular basis of myocardial vulnerability. In this context, measuring coronary flow reserve and coronary endothelial function may be more useful than brachial artery measures to predict and assess potential damage related to limited vascular responsiveness. This review aims to provide a short overview of coronary flow reserve, its different modalities of measurement, as well as its utility in cardiovascular research.

PHYSIOLOGICAL CONCEPT OF CORONARY FLOW RESERVE

The concept of coronary flow reserve was introduced by Coffman and Gregg [12], who described the capacity of the coronary circulation to conduct maximal hyperaemic blood flow. However, it was the pioneering research efforts of Dr Lance Gould who explored the relationship between the anatomic severity of a stenosis and its flow resistance [13]. In 1974, Gould and colleagues revolutionized our understanding of coronary physiology by demonstrating that resting myocardial blood flow remains normal until the epicardial coronary artery is stenosed > 85 % and that hyperaemic blood flow reduces with > 50 % epicardial stenosis [13]. He defined coronary flow reserve as the ratio of hyperaemic flow to resting flow for a given artery and showed that coronary flow reserve decreases with increasing stenosis severity [13]. In 1990, he validated the concept of intracoronary Doppler ultrasound scanning-derived coronary flow reserve as a measure of lesion severity [14]. Because blood velocity should be proportional to flow for a constant vessel area, coronary flow reserve could be calculated from the hyperaemic flow divided by resting blood velocity in a vessel.

The physiology of coronary circulation can be appreciated in terms of the flow–pressure relationship [5]. At rest, the coronary blood flow is generally regulated by the determinants of myocardial oxygen demand (heart rate, contractility and ventricular wall tension). When myocardial oxygen demand is constant, coronary blood flow is subjected to autoregulation: a relatively large change in perfusion pressure will cause only minor variation to the blood flow [5]. However, when exposed to maximal vasodilation, the coronary blood flow escapes from autoregulation and exhibits a linear relationship with the myocardial perfusion pressure (Figure 1). Under
normal conditions, the flow to the heart is controlled mainly by constriction and dilation of the microcirculation (vessels less than 400 μm in diameter). At rest, an increase in coronary blood flow occurring with augmented myocardial oxygen demands is regulated by the changes in the vascular resistance of both the epicardial arteries and the coronary arterioles. The arterioles provide the greatest resistance to flow. The resistance of the larger epicardial coronary arteries visible on a coronary angiogram is almost negligible in the absence of a stenosis. In the presence of a stenosis in an epicardial artery, a fall in pressure across the stenotic lesion occurs and the microvessels dilate to compensate for the reduced distal arterial perfusion pressure, maintaining normal resting flow. However, if vasodilation is induced, the ability for the already dilated microcirculation to reach maximal dilatation is reduced. This concept is physiologically important in determining the functional significance of a stenosis or the dysfunction in the vasodilatory capacity of coronary microcirculation. The difference between maximal coronary blood flow and resting blood flow for a given perfusion pressure corresponds to the coronary flow reserve (Figure 1). Hence coronary flow reserve can be regarded as the ability to increase coronary blood flow in response to vasoactive mechanism.

The maximal flow response can be achieved by physiological stimuli (dynamic exercise, transient vessel occlusion or atrial pacing) or pharmacological agents. Pharmacological stimulation of microvascular vasodilation has been considered the most appropriate for the clinical setting. Several pharmacological agents may be applied either intravenously or by direct intracoronary injection [15,16]. Despite the wide application, the unique characteristics of each agent must be considered when designing or interpreting a clinical study [15,16].

Papaverine is the gold standard for producing maximal dilatation, which can be achieved with intracoronary doses of 8–12 mg. It reaches its maximal effect within 10–20 s and its action lasts approx. 120 s. However, there are three major concerns regarding its use. First, in contrast with the intracoronary administration, intravenous administration causes severe arterial hypotension. Secondly, metabolism and excretion of papaverine are slow, and systemic half-life is in the range of 3.5 h. Thirdly, papaverine lengthens the QT interval and may cause torsades de pointes tachycardia in approx. 1% of the patients requiring electrical cardioversion [17]. Arrhythmias are facilitated by co-existing hypokalaemia, hypomagnesaemia, and alkalosis.

Dipyridamole causes a prolonged maximal coronary dilatation in approx. 2–4 min, and blood flow levels remain elevated for more than 30 min [18]. However, it should be noted that the standard dosage of dipyridamole (0.56 mg/kg of body weight infused intravenously over 4 min) does not produce consistently maximal vasodilation in all individuals [19]. In addition, the intake of coffee within 1 h before dipyridamole infusion reduces maximal hyperaemic flow, because of the drug interaction with methyl xanthine derivatives [20]. A further limitation of dipyridamole is the potential induction of coronary steal phenomena, which may alter coronary flow reserve assessment.

Adenosine reliably causes maximal hyperaemia. Its action lasts for only 40 s. Dose-response studies have demonstrated that bolus injections of more than 16 μg into the left coronary artery and more than 12 μg into the right coronary artery caused maximal coronary vasodilation in 91% of the patients without significantly changing heart rate, atrioventricular conduction or perfusion pressure [21]. Adenosine may also be administered by continuous intravenous infusion. In our experience, intravenous adenosine has been used in conjunction with transthoracic Doppler echocardiography in the determination of coronary flow reserve [22–24]. Our infusion protocol involves administering ATP (0.14 mg · min⁻¹ · kg⁻¹ of body weight) intravenously for at least 2 min to record spectral Doppler signals. To date, we have not experienced any untoward side effects with respect to conduction abnormalities, such as sinoatrial and atrioventricular node depression. The most common complaint has been flushing. At present, adenosine is the agent of choice for repetitive measurements during diagnostic and interventional procedures.

MEASUREMENT OF CORONARY FLOW RESERVE

Coronary flow reserve depends on at least three factors: (i) the vascular resistance of the small and large coronary arteries (vascular component), (ii) the myocardial resistance (extravascular component), and (iii) factors that affect the composition of blood (rheologic component). Thus abnormal coronary flow reserve may be the result of alterations in resting and/or in maximal flow with a compromise of one or more of the three component factors.

Coronary flow reserve can be expressed in terms of an absolute or relative value, based on flow velocity or pressure measurement. Absolute CVR (coronary velocity reserve) is defined as the ratio of hyperaemic to basal mean flow velocity.

The normal values for CVR vary according to the different methods of measurement. The CVR normal range varies from 2.5–5.5 when measured by densitometric analysis of coronary angiograms [15,16] or PET (positron emission tomography) [15,16], and between 1.8–5.3 with intracoronary Doppler flow measurements [15,16]. A normal absolute CVR indicates a normal twocomponent system, with a patent epicardial conduit supplying a normal myocardial bed. In the absence of epicardial conduit obstruction, the CVR may be abnormal.
when the microvascular circulation is compromised by LV (left ventricular) hypertrophy, coronary endothelial dysfunction or other diseased rheological conditions [25,26].

However, when coronary stenosis is present, additional measurement of CVR in an adjacent normal vessel may be needed as a reference value to discriminate between the two vascular entities. An abnormal CVR ratio between target vessel to angiographically normal reference coronary artery, i.e. rCVR (relative CVR), enhances the contribution of the stenosis to the impairment of flow. A relative coronary flow reserve value of more than 0.8 has been described as signifying normal coronary flow [27]. However, evidence exists that angiographically normal contralateral arteries of patients with known atherosclerosis have abnormal flow [28].

It should be noted that coronary flow reserve may vary with haemodynamic changes [29]. FFR (fractional flow reserve) was developed as a pressure-derived index of epicardial stenosis severity, inclusive of collateral flow, theoretically independent of baseline haemodynamics, and with an unequivocal normal value of 1 [28,30,31]. FFR is the fraction of maximal coronary blood flow that goes through a stenotic vessel expressed as percentage of blood flow through the same artery in the theoretical absence of the stenosis. FFR is inversely derived and requires a pressure wire advanced through a guiding catheter within the coronary artery. Mean aortic pressure (through the guiding catheter) and mean distal pressure (with the pressure wire) are recorded at baseline and maximum hyperaemia. It is calculated as the ratio of the absolute distal coronary and aortic pressures measured during maximal hyperaemia. In the presence of a coronary artery stenosis, the larger the imposed resistance caused by greater area of stenosis, the larger the fall in pressure along the vessel and, therefore, the smaller the FFR obtained. Conversely, in normal arteries, there is no pressure drop and FFR = 1.

Critique of the FFR theory centres around concern regarding the pressure derivation of the FFR, which may inaccurately reflect actual flow impedance, especially in diseased hearts in which the myocardial resistance may not be constant or uniform. Critics note that there is significant discordance between coronary flow reserve and FFR, especially in patients with diseased microvascular pathology [32]. Pressure distal to a stenosis depends on flow across the stenosis, which is determined with both microvascular and epicardial resistance. A full understanding of a patient requires definition of both epicardial and myocardial resistance to flow. FFR combined with coronary flow reserve, which reflects microvascular and epicardial resistance, may deliver a more complete picture. The necessity of both a pressure wire and a Doppler ultrasound scanning-tipped wire has limited this approach. Recently, a novel measurement of pressure-derived coronary flow reserve has been evaluated [33]. The square root of the hyperaemic translesional gradient divided by the square root of the resting translesional gradient correlated with the Doppler ultrasound scanning-derived coronary flow reserve in moderate stenoses [33]. This measurement may enable simultaneous evaluation of coronary flow reserve and FFR, yielding epicardial and microvascular information with a single pressure wire [34]. More progress is clearly necessary in this field.

**MEASUREMENT OF CORONARY ENDOTHELIAL-DEPENDENT VASOMOTOR FUNCTION**

In the determination of coronary flow reserve, the vasodilators papaverine, adenosine and dipyridamole are all direct endothelium-independent vascular smooth muscle dilators and provide information on coronary flow reserve and the predominantly vascular smooth muscle control.

With respect to coronary endothelium-dependent vasoreactivity, this can be tested by using different stimuli involving both invasive and non-invasive modalities. Until recently, the evaluation of endothelium-dependent coronary vasoreactivity in humans focused primarily on the intracoronary administration of acetylcholine, bradykinin or substance P. Acetylcholine, the classic stimulus for endothelium-mediated relaxation, acts via muscarinic membrane receptors with signal transduction through G-proteins to mediate the release of the predominantly relaxing factor NO, as well as an endothelium-derived hyperpolarizing factor that counteracts the direct vasoconstrictor effects of acetylcholine via muscarinic receptors on the smooth muscle layer. A vasodilator response to acetylcholine indicates preserved endothelial vasodilator function [35]. However, recent studies have demonstrated that abnormal endothelial-dependent vasoreactivity of conduit and resistance vessels in response to acetylcholine reveals a significant disparity [36]. Whether this applies to a physiologically more relevant endothelial function test, such as sympathetic activation by cold pressor testing, remains to be determined.

Besides pharmacological stimuli, such as with acetylcholine, physical stimuli may also be used to assess coronary endothelial vasoreactivity. Sympathetic activation by cold pressor testing integrates the effects of adrenergic receptor stimulation in both the endothelium and smooth muscle cell layer and flow-dependent epicardial vasodilation secondary to increased coronary blood flow due to augmented myocardial demand [37,38]. Previous studies have demonstrated that coronary vasomotor responses to the sympathetic activation induced by mental stress or cold pressor testing correlate closely with vasomotor responses to acetylcholine [35,40].
suggesting that a coronary vasodilator response to sympatheic activation reflects the functional integrity of the endothelium [41]. More recently, these stressors have tested with non-invasive PET [42,43] and with trans-thoracic Doppler echocardiography [44]. However, one drawback with these adrenergic stressors is that an impaired efferent adrenergic system [45] and concurrent adrenergic modulating therapies such as adrenergic blockers may alter the coronary flow responses. Clearly, the characteristics of the stressor must be considered when designing or interpreting a clinical or research study.

Nevertheless, it could be argued that any research study aiming to fully assess the global parameter and integrated function of coronary circulation may require the measurement of both coronary flow reserve and of endothelium-dependent vasodilation. The recent development of various non-invasive methods of coronary flow may allow its serial measurements.

**METHODS OF CORONARY FLOW MEASUREMENT**

There are a number of different approaches and techniques which can be used for the assessment of coronary flow reserve. Invasive methods of coronary flow reserve measurement are usually carried out in the cardiac catheterization laboratory. Miniaturized sensors can nowadays be mounted on angioplasty guide wires to assess pressure, flow and temperature, which facilitate the determination of coronary flow reserve by invasive techniques such as coronary sinus thermodilution, diffusible indicators, angiographic densitometry or intracoronary Doppler flow measurement. Although these methods have been validated in human studies [46,47], their invasive nature means that they are only available during cardiac catheterization and generally do not allow serial measurements of coronary flow reserve. Therefore invasive methods have limited applicability for large-scale studies or future development as a clinical tool.

**NON-INVASIVE MEASUREMENT OF CORONARY FLOW**

The non- or semi-invasive approaches to coronary imaging and flow determination have included PET, MR (magnetic resonance) imaging and new echocardiography techniques in TEE (transoesophageal echocardiography), myocardial contrast echocardiography and transthoracic Doppler echocardiography. However, it should be noted that these methodologies are, as yet, experimental and each have limitations, primarily related to sampling feasibility or resolution.

**PET**

PET can determine regional blood flow per unit mass of myocardium in as little as 10 g of myocardium and is able to provide data on myocardial metabolism [16]. In this respect, PET is able to match myocardial metabolism with perfusion. However, it should be noted PET is not able to determine the transmural flow distribution. A number of different perfusion tracers administered intravenously have been used which utilizes macro-aggregated albumin microspheres labelled with 68Ga, 11C, [13N]ammonia, [15O]water and 82Rb. Flow estimation by [13N]ammonia PET has been validated in human studies [48,49], which confirmed its accuracy in determining global myocardial flow in both normal and disease states even without epicardial coronary artery disease. Coronary flow can also be accurately measured with [15O]water PET after dipyridamole administration in patients before and after angioplasty [50]. This tracer allows the simultaneous measurement of myocardial viability and flow with a pixel-to-pixel subtraction technique and can potentially discriminate between ‘hibernating’ myocardium and postinfarction scars. In brief, PET provides accurate measurements of both myocardial flow and metabolism; however, PET is expensive, time consuming and is not widely available. This has limited the widespread clinical application of PET assessments of coronary flow reserve.

**MR imaging**

MR imaging has a unique potential for non-invasive measurement of volumetric coronary blood flow in the proximal and distal portions of the three major coronary arteries. MR blood flow quantification in the coronary artery has been very challenging because the coronary artery is small (< 3–4 mm), and is subject to both cardiac and respiratory motions. Several different approaches have been proposed for quantifying coronary blood flow. Currently, the phase-contrast technique is the most frequently used technique for MR flow measurement in the coronary vessels [51]. Phase-contrast velocity-encoded cine MR imaging can provide flow measurements at multiple temporal phases in the cardiac cycle. Diastolic peak velocities in the LAD (left anterior descending) artery in the basal state and after intravenous dipyridamole have demonstrated an average coronary flow velocity reserve in eight normal subjects to be $3.14 \pm 0.59$ [52]. There are several sources of errors with MR imaging. Compromise of the spatial resolution of MR imaging in comparison with the vessel size may lead to an overestimation of blood flow volume. If the data acquisition window in the cardiac cycle is insufficient, blurring of the vessel may result in inaccurate flow velocity and volume measurements. However, the use of a stronger gradient system designed for ultrafast cardiac MR imaging can improve the accuracy of MR flow and newer faster scan techniques, including spiral...
scanning, are expected to make the acquisition duration time shorter.

**New ultrasound technology**

Recently, non-invasive studies using new ultrasound technology and contrast echocardiography have allowed coronary blood flow in the epicardial vessels and microcirculation to be observed, providing the potential for the complete non-invasive assessment of the structure, physiology and pathophysiology of the coronary circulation.

**Myocardial contrast echocardiography**

Recent studies [53,54] using myocardial contrast echocardiography with new ultrasound technology have allowed coronary blood flow in the epicardial vessels and microcirculation to be observed. Doppler signal from coronaries can be enhanced by using intravenously injected contrast agents that survive transpulmonary passage. Harmonic Doppler can further enhance Doppler signal-to-noise ratio by reducing the noise coming from the tissue without reducing Doppler signal intensity. Indeed, harmonic imaging enhancement of intravenously administered ultrasound contrast in the LV myocardium has provided non-invasive observation of blood flow within epicardial and intramyocardial arteries. Early canine studies that used contrast-enhanced Doppler recordings from within intramyocardial coronary arteries demonstrated primarily diastolic flow velocities consistent with the known flow pattern of coronary arteries [53]. Clinically, Caiati and co-workers [54] have demonstrated that transthoracic harmonic colour Doppler and pulse-wave Doppler in conjunction with intravenous injection of an echocardiographic contrast agent with appropriate ultrasound characteristics (Levovist; Schering AG, Berlin, Germany) can reliably evaluate blood flow velocity in the LAD artery at rest and during hyperaemia (induced by intravenous adenosine). In a small series of 25 patients, they [54] showed that coronary flow reserve in the LAD artery, as assessed by contrast-enhanced transthoracic Doppler echocardiography along with harmonic mode, concurred very closely with Doppler flow wire coronary flow reserve measurements. It should be noted that myocardial contrast echocardiography is a rapidly developing technology and that there is continuing investigation of extended applications of existing contrast agents, development of newer contrast agents and optimization of ultrasound imaging acquisition techniques which will determine further its application in assessing coronary vasomotor function.

**TEE**

TEE allows the visualization of the proximal segments of coronary arteries (generally the left main trunk and the first centimeters of the circumflex arteries and anterior descending artery). However, most studies of coronary flow velocity have been of Doppler recordings of coronary blood flow velocity in the LAD artery. TEE Doppler has also been used with both dipyridamole and adenosine as coronary vasodilators [55,56]. Flow velocity may be underestimated because of the angle between the ultrasound beam and the vessel and because of changes in arterial diameter during hyperaemia. Biplane or multi-plane TEE Doppler and the availability of vessel tracking Doppler systems have improved further the accuracy of this technique. TEE Doppler is relatively easy and safe to perform and may become an important tool for assessing coronary flow reserve in the LAD artery. The limitations of TEE Doppler are that it is highly dependent on a good Doppler signal and only the LAD artery can be studied and, arguably, it is a semi-invasive procedure which might limit its widespread research applications.

**Transthoracic Doppler echocardiography**

The development of new high frequency, high resolution ultrasound equipment has enabled the clinical detection of the mid-to-distal LAD coronary artery using transthoracic colour flow Doppler [57–59]. Using this technology, imaging of the LAD artery and perforating branches and measurement of coronary blood flow has been carried out at rest and after pharmacological alterations (sublingual nitroglycerine and intravenous adenosine) in patients without and with significant LAD artery stenosis. These studies [57–59] report a variable success rate in obtaining coronary flow velocities from 44–94%.

It has been noted that a highly experienced operator is required to achieve a sufficient success rate in measuring coronary flow velocity with transthoracic Doppler. We have significant experience with this technique and believe that, after an initial learning curve, it is possible to study coronary flow in the LAD artery in more than 75% of cases. In brief, we use a 7.0 MHz transducer (Acuson Sequoia 512; Siemens Medical Solutions USA, Mountain View, CA, U.S.A.). In colour Doppler flow mapping, the velocity range is set in the range of ± 12 cm/s. The colour gain is adjusted to provide the optimal images. The ultrasound beam is transmitted towards the heart to visualize coronary blood flow in the LAD artery by colour Doppler echocardiography. First, the left ventricle is imaged in the long-axis cross section, and the ultrasound beam is inclined laterally. Next, coronary blood flow in the distal portion of the LAD artery is searched under the guidance of colour Doppler flow mapping. With a sample volume (2.5–3.0 mm wide) positioned on the colour signal in the LAD artery, Doppler spectral tracings of flow velocity in the LAD artery (Figure 2) is recorded by fast-Fourier-transformation analysis. Although we try to align the ultrasound beam direction to the distal LAD artery flow as parallel as possible, angle correction is generally needed in each examination because of incident Doppler angle (mean angle, 45°;
range, from 31–58°). We first record baseline spectral Doppler signals in the distal portion of the LAD artery over five cardiac cycles at end-expiration. Intravenous adenosine is then administered (140 µg·min⁻¹·kg⁻¹ of body weight, intravenously) for 2 min to record spectral Doppler signals during hyperaemic conditions (Figure 2). MDV (mean diastolic velocities) are measured at baseline and at peak hyperaemic conditions from the Doppler signal recordings. Measurements are then averaged over three cardiac cycles. Coronary flow velocity reserve is defined as the ratio of hyperaemic to basal MDV. At present, this methodology is subject to the inherent limitations and assumptions of the Doppler technique and also to the limited access to coronary vessels other than the LAD artery. Without estimation of the coronary artery diameter, the technique only allows measurement of coronary flow velocity, but not changes in coronary flow. However, it has been shown that coronary flow reserve measured using both parameters is closely related [60] and in large invasive studies, such as DEBATE (Doppler Endpoints Ballon Angioplasty Europe) [61] and DESTINI (Doppler Endpoint Stenting International Investigation) [62], the coronary flow reserve derived from changes only in velocity of coronary blood flow is accepted instead of the absolute blood flow, but provides an exciting possibility for the non-invasive physiological assessment of the coronary vasculature at rest and during stress. Serial assessment can be performed by this technique because of its non-invasive and relatively inexpensive nature.

**UTILITY OF CORONARY FLOW MEASUREMENT IN RESEARCH**

In the clinical setting, coronary flow reserve has been used in complementing coronary angiography for better assessment of the severity of coronary artery disease, selection of patients for appropriate interventional therapy, as well as determining the functional result of angiography and/or coronary stenting. Measurement of coronary flow reserve also facilitates in clinical decision-making on coronary bypass surgery in patients with equivocal left main coronary artery disease. It has also been used increasingly to assess the functional significance of cardiac allograft vasculopathy after transplantation. The clinical application of coronary flow reserve measurement has been reviewed extensively elsewhere [15,16]. In this review, we will focus on the research potential of coronary vasomotor function testing.

In the recent years, there has also been a growing interest on the role of coronary flow measurement in assessing the coronary endothelial function and coronary microvascular function. Measurement of coronary flow has been utilized in research to assess coronary microvascular function in the absence of overt coronary stenosis. Determination of coronary flow measurements employing coronary vasodilators provides important information on coronary flow reserve and the predominantly vascular smooth muscle control. With respect to endothelial-dependent vasodilation, adrenergically mediated flow responses to cold pressor testing and hand-grip [35,40] have been tested using non-invasive PET measured myocardial blood flow [42,43] and transthoracic Doppler echocardiography [44]. It has been argued that any research study aiming to fully assess the global parameter and integrated function of coronary circulation may require the measurement of both coronary flow reserve and endothelium-dependent vasodilation. Studies to date have generally assessed the impact of cardiovascular risk factors on coronary vasomotor function.

**Effects of age and sex on coronary flow**

There is a reduction in coronary flow reserve in older patients [63]. The reduction is mainly attributed to the increase in baseline flow caused by an increased rate-pressure product in older patients, because hyperaemic flows are similar [63]. These findings suggest that reduction in coronary flow reserve with age is primarily caused by an increased cardiac workload and blood flow at rest rather than an abnormal maximal flow. Coronary flow reserve is generally similar in men and women [63].

**Coronary flow and diabetes mellitus**

Diabetes mellitus predisposes people to premature atherosclerotic coronary artery disease and, for this reason, coronary flow abnormalities in diabetes mellitus have been studied extensively. There is consistent evidence that coronary vascular function is impaired in patients with diabetes and that this precedes clinically overt coronary artery disease, suggesting that it may be an early marker.
of atherosclerosis [64,65]. Coronary flow reserve has consistently been shown to be reduced in diabetic patients with angiographically normal epicardial arteries [64,65]. Moreover, we have shown previously [66] that reduction in coronary flow reserve is more marked in patients with diabetic retinopathy. Factors such as diabetes-associated hypertension and dyslipidaemia may contribute to the severity of vascular dysfunction in diabetes. However, there is evidence to show that, in diabetes, coronary vascular dysfunction is present even in subjects without hypertension or dyslipidaemia, suggesting that diabetes or a constellation of factors associated with it (e.g. hyperglycaemia and insulin resistance) may be causally related to this vascular dysfunction [67]. The mechanism for this may be related to a reduction in the maximal area of the coronary microvessels and limited maximal hyperaemic flow due to both wall thickening and lumen narrowing of intramural coronary microvessels in patients with diabetes mellitus. However, coronary endothelial dysfunction may also contribute to this vascular dysfunction. In this respect, Di Carli and co-workers [68] utilized PET to measure myocardial blood flow at rest, during adenosine-induced hyperaemia (reflecting primarily endothelium-independent vasodilation) and in response to cold pressor test (reflecting primarily endothelium-dependent vasodilation) in young subjects with both Type I and Type II diabetes mellitus and in age-matched healthy controls. Myocardial vasodilator reserve was reported to be reduced by 54 % and 47 % in Type I and II diabetics respectively, compared with controls. Likewise, the myocardial blood response to cold was reduced by 65 % and 71 % in Type I and Type II diabetics respectively, compared with controls. These observations suggest a key role of coronary endothelial dysfunction in the pathogenesis of vascular dysfunction in diabetes. More recently, it has been shown [69] that coronary vasomotor dysfunction may even be present in insulin-resistant patients who are otherwise healthy. In a study involving a single ethnic sample of Mexican–American participants who were insulin-resistant but do not have hypercholesterolaemia or hypertension and did not smoke, Quinones and co-workers [69] demonstrated a 70 % reduction in myocardial blood flow responses to cold pressor test in insulin-resistant patients. This abnormal cold pressor test response occurred despite a normal flow response to dipyridamole, suggesting that it is a potential abnormality of coronary endothelium in these patients. Interestingly, thiazolidinedione therapy in a subgroup of insulin-resistant patients normalized coronary endothelial function, although it should be noted that this was an open-label non-randomized study [69]. Besides studies examining coronary flow as a marker of early atherosclerosis, there have been studies examining the relationship between coronary flow and cardiac function in diabetes, and these studies have revealed a significant reduction in coronary reserve which was associated with an impairment of diastolic function (increased relaxation time index and reduced diastolic in-flow pattern) [70]. This may provide evidence to suggest that functional coronary microvascular abnormalities, as reflected by reduction in coronary flow reserve, may be implicated as the preliminary process in the pathophysiology of diabetic cardiomyopathy.

**Coronary flow and hypercholesterolaemia**

Hypercholesterolaemia is known to be associated with functional and structural abnormalities that extend to the coronary microvasculature. Coronary flow abnormalities have been demonstrated consistently in patients with hypercholesterolaemia [71,72]. The relationship of in vivo LDL (low-density lipoprotein) oxidation and coronary reactivity in young men was studied by Raitakari and co-workers [73]. Coronary flow reserve was found to be inversely associated with auto-antibody titre against ox-LDL (oxidized LDL; a marker of in vivo LDL oxidation), supporting the concept of direct coronary endothelial damage through LDL oxidative mechanisms. There were also significant inverse correlations between coronary flow reserve and the total plasma cholesterol as well as plasma LDL-cholesterol [73]. Coronary endothelial dysfunction has also been consistently demonstrated in experimental settings [74,75]. Reduced bioavailability of NO in human coronary epicardial and microvessels in the basal condition and during pharmacological stimulation has been demonstrated [75]. An improvement in coronary flow reserve by normalizing the plasma cholesterol level within 24 h after a single LDL aphaeresis has been shown [76]. Studies have also shown improvement in coronary vasodilatory capacity following lipid-lowering therapy in hypercholesterolaemia. We have shown recently [77] that coronary flow reserve improved following therapy for 3 months with fluvastatin in patients with hypercholesterolaemia. Diets containing high levels of lipid oxidation products may induce endothelial dysfunction. In this respect, we demonstrated recently [78] an 18 % reduction in coronary flow reserve following a single high fat meal in young healthy men.

**Coronary flow in hypertension and LV hypertrophy**

Coronary vasomotor dysfunction has been described in patients with essential hypertension and hypertensive heart disease [79]. The presence of concomitant LV hypertrophy in hypertensive patients is a strong predictor of cardiovascular morbidity and mortality and this may be related to its impact on coronary vasomotor function. A recent study [80] demonstrated that, in hypertensive patients, increased LV mass was higher than needed to compensate for increased cardiac workload at a given body size. This excessively increased LV
mass was associated with lower coronary vasodilator capacity, more depressed LV wall mechanics and abnormal LV diastolic filling pattern [80].

The underlying mechanisms are not yet fully understood; however, structural as well as functional alterations in the sense of microvascular disease in the coronary resistance vessels appear to play a major role. In patients with asymptomatic borderline hypertension without evidence of LV hypertrophy, coronary flow reserve was found to be reduced, mainly due to impaired maximal vasodilatory capacity [81]. Data from myocardial biopsies indicate that the increased coronary resistance in hypertensive patients is related to structural changes, such as mean external arteriolar diameter, mean arteriolar wall area, percentage medial wall area, mean periarteriolar fibrosis area and volume density of total fibrosis [82]. On the other hand, the reduction in coronary flow reserve may, in part, be explained by an increase in resting blood flow. In comparisons of hypertrophied and non-hypertrophied left ventricles, the reduction in coronary flow reserve is predominantly caused by an increase in baseline flow velocities [83]. In addition, ventricular dilation associated with increased LV end-diastolic pressures appears to play a more important role than ventricular hypertrophy alone [84]. Both the increased myocardial component of coronary resistance and the increased wall stress appear to be responsible for coronary flow reserve reduction. Clinical as well as experimental data have demonstrated that these structural changes may be reversible with antihypertensive treatment within 12 months and that improvement of coronary flow reserve may be related to a regression in smooth muscle vascular hypertrophy in the coronary resistance vessels [85].

The influence of hypertrophy on coronary vasomotor function has not been fully defined. This controversy is, in part, related to different causes of cardiac hypertrophy, such as primary hypertrophy (as in hypertrophic obstructive cardiomyopathy), reactive hypertrophy (as in aortic stenosis) and hypertensive hypertrophy. Although LV hypertrophy is associated with a reduction in coronary flow reserve and coronary endothelial function [86], LV hypertrophy, as assessed by LV mass-to-volume ratio, does not correlate well with the reduction in coronary flow reserve [79]. Data from patients with hypertrophic obstructive cardiomyopathy support this hypothesis. Microvascular changes occur not only in the most hypertrophied regions of hypertrophic obstructive cardiomyopathy, but also in areas with minimal or no hypertrophy, suggesting genuine structural abnormalities [87]. The importance of the pressure gradient across the aortic valve on coronary flow reserve, however, should not be underestimated, because the removal of the obstructive aortic valve does not improve the decreased coronary flow reserve immediately after aortic valve replacement [88]. In addition, changes in basal coronary flow must be considered in patients with altered intraventricular pressure gradients [89].

**Effects of other cardiovascular risk factors on coronary flow**

Our laboratory has been utilizing transthoracic colour Doppler echocardiography to investigate the impact of a number of cardiovascular risk factors on coronary vasomotor function and have found it to be particularly useful for serial measurements which has allowed us to investigate the acute impact of various therapeutic manoeuvres. For example, we have shown [23] that an acute 30 min exposure in a smoking room reduced coronary flow velocity reserve by 23 % in non-smokers (Figure 3). In postmenopausal women, we have shown [24] that, 2 h following administration of a conjugated oestrogen, coronary flow velocity reserve increased by 21 % (Figure 4), and in healthy volunteers, acute black tea consumption improved coronary flow velocity reserve [90].

**Coronary flow and prognostic importance**

The clinical relevance of coronary flow measurement is dependent to some extent on its prognostic importance. In this respect, Schachinger and co-workers [91] have investigated prospectively whether coronary endothelial dysfunction predicted disease progression and cardiovascular events. Coronary vasoreactivity was assessed invasively in 147 patients undergoing either routine diagnostic catheterization for evaluation of chest pain or angioplasty, using the endothelium-dependent dilator acetylcholine, cold pressor test and dilation in response to nitroglycerine. Over a follow-up period of
Individual changes in coronary flow velocity reserve (CFVR) at baseline and after administration of conjugated oestrogen

7.7 years, all tests of coronary vasoreactivity were found to be significant independent predictors of a poor prognosis, even after adjustment for traditional cardiovascular risk factors or the presence of atherosclerosis itself. It should be noted that there is no prognostic information with the newer non-invasive techniques, such as with PET or Doppler echocardiography.

CONCLUSIONS
Coronary flow reserve and coronary endothelial function is a useful concept that can be applied to study the pathophysiology of coronary circulation. It can provide integrative information on the functional importance of epicardial stenosis as well as the coronary microcirculation. An appropriate interpretation of coronary vasomotor function is deemed necessary to accurately evaluate myocardial perfusion in various populations. Currently, there are many different approaches used to assess coronary flow. Invasive techniques have been proven to be sensitive and specific, but can only be applied during cardiac catheterization, limiting their practical use in routine clinical practice. The use of non-invasive methods, such as PET, for coronary flow measurement has gained substantial credit in clinical practice. However, they are relatively expensive and not widely available. Transthoracic Doppler echocardiography has been proven to be a practical and relatively inexpensive method to assess coronary function in a totally non-invasive way. However, the prognostic importance of coronary vasomotor function has only been shown with invasively derived coronary vasoreactivity and not with the non-invasive methods. The same rigorous evaluation is needed to determine the prognostic value of these newer non-invasive measures of coronary vasomotor function.

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