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Update on Nitric Oxide-Dependent Vasodilation in Human Subjects

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Abstract

There currently is great interest in translating findings about the importance of nitric oxide (NO) in vascular biology to the Clinical arena. The bioactivity of endothelium-derived NO can readily be assessed in human subjects as vasodilation of conduit arteries or increased flow, which reflects vasodilation of resistance vessels. This chapter provides an update on the available noninvasive methodology to assess endothelium-dependent vasodilation in human subjects.

Background

A vast body of work has emphasized the importance of endothelium-derived nitric oxide (NO) in vascular biology, and there currently is great interest in translating these findings to the clinical practice. Because it is a potent vasodilator, the bioavailability of endothelium-derived NO can readily be evaluated in human subjects by measuring changes in arterial diameter and blood flow. Human studies have shown that impaired endothelium dependent vasodilation is associated with the presence of atherosclerosis and recognized cardiovascular disease risk factors. Many interventions that reduce cardiovascular risk also restore endothelium-dependent vasodilation toward normal. Importantly, prospective studies have shown that the presence of impaired endothelium-dependent vasodilation in the coronary or peripheral circulation identifies patients with increased risk for future cardiovascular disease events (Widlansky *et al.*, 2003). In a review of methods for measurement of NO-dependent vasodilation in humans. Vita (2002) described invasive methods for the study of the coronary and peripheral circulations and use of two-dimensional ultrasound to study flow-mediated dilation of the brachial artery. Since that time, additional noninvasive approaches have emerged for study of NO-dependent control of vascular tone in the coronary circulation, central aorta, and peripheral microcirculation. In this chapter, we briefly mention advances in the previously described methods (Vita, 2002) and then describe these newer methods for assessment of NO-dependent vasodilation in humans (Table I).

Studies of the Coronary Circulation

Invasive Studies

A number of studies have examined NO-dependent vasodilation in the coronary circulation of patients undergoing cardiac catheterization. These studies assess changes in coronary artery diameter or coronary blood flow during intraarterial agonist infusion using quantitative coronary angiography and intracoronary Doppler, respectively (Vita, 2002). Though generally extremely safe, these studies have the potential to produce major complications such as coronary thrombosis or death. Because of their invasive nature, they are not well suited for repeated studies in the same individual or for the study of relatively low-risk populations. Despite these limitations, studies of the coronary circulation are the most clinically relevant for coronary artery disease. In particular, studies have shown that abnormalities of NO-dependent vasodilation in the coronary circulation are associated with increased risk for

cardiovascular disease events (Halcox *et al.*, 2002; Schachinger *et al.*, 2000; Schindler *et al.*, 2003; Suwaidi *et al.*, 2000; Targonski *et al.*, 2003).

Noninvasive Studies

Given its clinical relevance, it would be desirable to obtain information about endothelium-dependent vasodilation of the coronary circulation in a noninvasive manner. Several studies have used transthoracic Doppler echocardiography to assess coronary blood flow reserve in left anterior descending (LAD) circulation. This method involves obtaining Doppler flow signals of the distal LAD using an acoustic window near the midclavicular line in the fourth and fifth intercostals spaces. Signals are recorded at baseline and then after 2 min of intravenous adenosine triphosphate (ATP) infusion (140 μg/kg/min) to increase coronary blood flow. Changes in coronary blood flow are expressed as the ratio of ATP-induced to basal coronary flow velocity (Olsuka *et al.*, 2001). Coronary "flow reserve" measured in this manner is acutely impaired by passive cigarette smoking and improved by interventions known to improve endothelium-dependent vasodilation (Hirata *et al.*, 2004; Otsuka *et al.*, 2001).

The methodology is limited as a test of endothelial vasodilator function, however, because the response to ATP is only partially dependent on endothelium-derived NO. Furthermore, systemic ATP infusion lowers blood pressure and increases heart rate, which may alter coronary blood flow independently of endothelial function. The technique is highly operator dependent. No published studies have evaluated the effect of inhibitors of NO synthase (NOS) on the response. Thus, the technique has potential as a noninvasive method for assessing NO-dependent and NO-independent vasodilation in the human coronary circulation, but further studies are required before it can be generally accepted. A number of other noninvasive methodologies also show promise for examination of endothelium-dependent changes in coronary blood flow, including magnetic resonance imaging and positron emission tomography but currently are not in use for this purpose.

Studies of the Arm and Hand

Invasive Studies

In light of the difficulty of studying the coronary circulation, many investigators have turned to the study of NO-dependent vasodilation in peripheral arteries. As reviewed by Vita (2002), studies of tins type involve infusion of various vasoactive drugs into the brachial artery and measurement of vasodilation as changes in forearm blood flow using venous occlusion plethysmography or changes in radial artery diameter using high-resolution vascular ultrasound (Creager *et al.*, 1990; Lieberman *et al.*, 1996). The clinical relevance of these studies is predicated on the assumption that many cardiovascular disease risk factors are systemic in nature and have parallel effects in different vascular beds. This assumption is strongly supported by studies showing that an impaired blood flow response to acetylcholine and other endothelium-dependent vasodilators is associated with increased risk for cardiovascular disease events (Fichtlscherer *et al.*, 2004; Heitzer *et al.*, 2001; Perticone *et al.*, 2001). Despite their clinical relevance, these studies require insertion of an arterial catheter, which reduces their applicability to the general population. Thus, the methodology remains extremely useful for studying selected populations and examining mechanisms of vascular dysfunction. However, there continues to be great interest in noninvasive methods to examine No-dependent vasodilation in the periphery.

Noninvasive Studies: Flow-Mediated Dilation

A widely used noninvasive method to assess endothelial vasomotor function is brachial artery flow-mediated dilation as assessed by ultrasound (Corretti *et al.*, 2002; Vita, 2002). In these studies, reactive hyperemia is induced by cuff occlusion of the arm, and changes in arterial

diameter are measured using high-resolution ultrasound (Corretti *et al.*, 2002). Flow-mediated dilation measured in this fashion depends on NO synthesis (Lieberman *et al.*, 1996), correlates with endothelial vasomotor function in the coronary circulation (Anderson *et al.*, 1995), and is reduced in the setting of traditional risk factors for coronary artery disease (Benjamin *et al.*, 2004). In addition, impaired brachial artery flow-mediated dilation predicts short-term and long-term risk for cardiovascular disease events in patients with advanced atherosclerosis (Gokce *et al.*, 2002) and in patients with hypertension (Modena *et al.*, 2002).

Despite its clinical relevance, ultrasound-based studies have a number of limitations. The technique is technically demanding, and changes in brachial diameter produced by hyperemic flow (0.1–0.6 mm) are close to the limit of detection of ultrasound. Reproduciability depends greatly on image quality, and the technique requires time-consuming off-line image analysis. For these reasons, investigators have sought new techniques that are faster and simpler to perform.

Noninvasive Studies: Pulse Amplitude Tonometry

One emerging method is known as fingertip pulse amplitude tonometry (PAT). Studies are performed using a commercially available device (Endo-PAT 2000, Itamar Medical, Ltd.) that records the pulse amplitude in fingertip at baseline and during reactive hyperemia. Hyperemia induces flow-mediated dilation within the fingertip and increases pulse amplitude. Simultaneous recordings are made from the contralateral finger and are used to adjust for changes in sympathetic tone and other systemic effects that might affect the signal during cuff occlusion and the hyperemic phase. Proprietary software provides further adjustment based on an empiric regression equation to account for baseline pulse amplitude, although the importance of making this adjustment remains unproven. The net response is expressed as the "reactive hyperemia PAT index." A preliminary study demonstrated that the increase in pulse amplitude is blocked, in part, by intraarterial infusion of monomethyl-L-arginine (L-NMMA), confirming that it depends in part on NO synthesis (Gerhard-Herman *et al.*, 2002). Interestingly, the reactive hyperemia PAT index has been reported to correlate with brachial artery flow-mediated dilation in the arm and is inversely related to risk factors and the presence of coronary artery disease (Kuvin *et al.*, 2003). The response also correlates with endothelial function in the coronary circulation (Bonetti*et al.*, 2004). Finally, the response improves after enhanced external counter pulsation therapy, an intervention known to improve peripheral artery endothelia] function (Bonetti *et al.*, 2003).

In our laboratory at Boston University School of Medicine, PAT and brachial ultrasound studies are done simultaneously using a single cuff occlusion to generate a period of reactive hyperemia, which stimulates flow-mediated dilation of both the conduit brachial artery and the small arteries in the finger. The PAT signals are recorded using thimble-shaped pneumatic probes that are placed on the index fingers of each hand. Patients lie supine with both wrists supported on foam blocks to allow the fingers to hang in an unsupported manner. The inflation pressure of the finger cuff is set to the diastolic pressure or 80 mm Hg (whichever is lower). Pulse recordings are made before cuff inflation and during the 1-min period beginning 1 min after 5-min cuff occlusion of the arm with the cuff placed on the upper arm. Figure 1 displays signals from a healthy subject and a subject with coronary artery disease. In a group of 252 unselected patients undergoing study of vascular function from our laboratory, the mean (±SD) reactive hyperemia PAT ratio was 2.2 ± 0.74 (range 1.23–5.69) with a highly skewed distribution. A prior study demonstrated that among patients referred for evaluation of chest pain, the reactive hyperemia PAT ratios were 1.31 ± 0.11 and 1.62 ± 0.47 for patients with and without exercise induced myocardial ischemia, respectively. We calculate that a sample size of 29 subjects per group would be required to detect a difference between groups of this magnitude with 80% power (alpha = 0.05) using log-transformed values for the reactive

hyperemia/PAT ratio. These results suggest that clinically important differences between study groups can be detected using this methodology in studies with samples sizes that are similar to those needed for study of brachial artery flow-mediated dilation (Vita, 2002). Overall, PAT appears to be a promising new methodology, but much work needs to be done to confirm its relation to other measures of NO-dependent vasodilation and to cardiovascular disease.

Noninvasive Studies: Extent of Reactive Hyperemia

Reactive hyperemia is the transient increase in limb blood flow that occurs after a period of limb occlusion and reflects ischemia-induced production of a variety of vasodilators, including adenosine and hydrogen ions that locally act on microvessels. A portion of the hyperemic response also depends on NO, possibly stimulated by local increases in shear stress during hyperemic flow. L-NNMA infusion blunts both the peak and the net hyperemic response in the forearm (Meredith *et al.*, 1996). Many investigators had suggested that reactive hyperemia is unaffected by cardiovascular disease. However, other studies have emphasized that reactive hyperemia is reduced in the setting of risk factors (Hayoz *et al.*, 1995; Higashi *et al.*, 2001; Mitchell *et al.*, 2004b) or coronary artery disease (Lieberman *et al.*, 1996), particularly the NO-dependent portion of the response (Higashi *et al.*, 2001). Reactive hyperemia also correlates inversely with systemic markers of inflammation, including C-reactive protein, interleukin-6, and the soluble form of intercellular adhesion molecule-1 (Vita *et al.*, 2004). Reactive hyperemia is the stimulus for brachial artery flow-mediated dilation, and we observed that a reduction in this stimulus accounts for much of the observed impairment in flow-mediated dilation observed in the setting of systemic risk factors (Mitchell *et al.*, 2004b). These findings suggest that noninvasive measures of flow can be used to assess reactive hyperemia as a clinically relevant correlate of endothelial vasomotor function.

We take two approaches to assessing reactive hyperemia. First, we use Doppler ultrasound to record flow signals from the brachial artery at baseline and for 15 s after cuff release after 5-min occlusion of the upper arm (Vita, 2002). Typical flow signals are displayed in Fig. 2. The peak hyperemic response is typically observed within two or three beats after cuff release. Images are digitized on-line, and we measure the average flow velocity (area under the curve) for the peak cardiac cycle using one of several image analysis software packages (Brachial Analyzer, Medical Imaging Applications, Iowa City, IA). Table II presents reference values from a cohort of 503 healthy subjects studied in our laboratory. Many investigators express hyperemic flow as the ratio of peak to baseline flow, but a recent study suggests that the hyperemic flow velocity and hyperemic shear stress (calculated from the velocity, brachial artery diameter, and assumed values for blood viscosity) correlate most strongly with cardiovascular disease risk factors and prevalent cardiovascular disease (Mitchell *et al.*, 2004b).

A second method to assess reactive hyperemic uses venous occlusion plethysmography to measure forearm blood flow before and after cuff release (Higashi *et al.*, 2001). Blood flow measurements are made using a mercury-in-silastic strain gauge, upper arm and wrist cuffs, and a computerized plethysmograph (Hokanson, Inc.) (Vita, 2002). During these studies, the upper arm venous occlusion cuff is inflated to 40 mm Hg (or adjusted to optimize the tracing), and circulation to the hand is excluded by inflation of the wrist cuff to suprasystolic pressure before initiation of flow measurements. At least five measurements are made and averaged at baseline, and a recording is made every 20 s after cuff release for 2 min. Although this methodology has limited ability to “capture” the peak flow response, it provides a reproducible approach to examine the entire hyperemic response.

Noninvasive Studies: Pulse Wave Analysis of Arterial Stiffness

There is great interest in examining arterial stiffness as a surrogate marker of atherosclerosis (Cohn *et al.*, 2004). A number of approaches can be used, including simple assessment of arterial pulse pressure measured by blood pressure cuff, pulse wave contour analysis assessed by tonometry, ultrasound visualization of arterial distensibility (calculated from the change in arterial diameter in relation to changes in blood pressure), and examination of pulse wave velocity. In regard to pulse wave velocity, a number of studies have shown that carotid-femoral pulse-wave velocity relates to cardiovascular disease risk factors and risk for future cardiovascular disease events (Cohn *et al.*, 2004). Whereas structural components of the arterial wall are major determinants of arterial stiffness, there is growing recognition that there also is a dynamic component of arterial stiffness that depends in part on arterial tone and endothelial release of NO. In support of the possibility, Wilkinson *et al.* (2002a,b)have observed that several measures of arterial stiffness are increased after systemic L-NMMA.

In our laboratory, we use applanation tonometry to assess vascular stiffness with a device developed at Cardiovascular Engineering, Inc. (Holliston, MA). Subjects lie quietly in a supine position, and pulse recordings are made from the carotid artery, brachial artery, radial artery, and femoral artery. Distances between recording sites are measured, and the pressures are calibrated using the brachial cuff pressure. Pulse recordings are gated using the electrocardiogram R-wave, and pulse wave velocity and the time of reflected waves are determined by blinded investigators. Reference values for a healthy, risk factor-free cohort were published this year (Mitchell *et al.*, 2004a). In some studies, we also made ultrasound recordings of flow and diameter of the left ventricular outflow tract, allowing us to calculate characteristic impendence, a variable that relates to stiffness of the proximal aorta (Mitchell *et al.*, 2002). One study demonstrated significant correlations between these measures of arterial stiffness and endothelial function (Nigam *et al.*, 2003), but further study will be required to define the precise contribute of endothelium-derived NO to arterial stiffness in different disease states.

Conclusions

The methodology for the study of NO-dependent vasodilation in intact humans continues to evolve. Many of the techniques are well established and have proven useful to study mechanisms of impaired NO bioavailability in atherosclerosis and related disease states and to evaluate potential therapies for these conditions. We suggest that some or all of these methods could be used clinically to assess cardiovascular risk or to guide risk-reduction therapy in individual patients. However, a great deal of work remains to be done to determine the clinical utility of these techniques.

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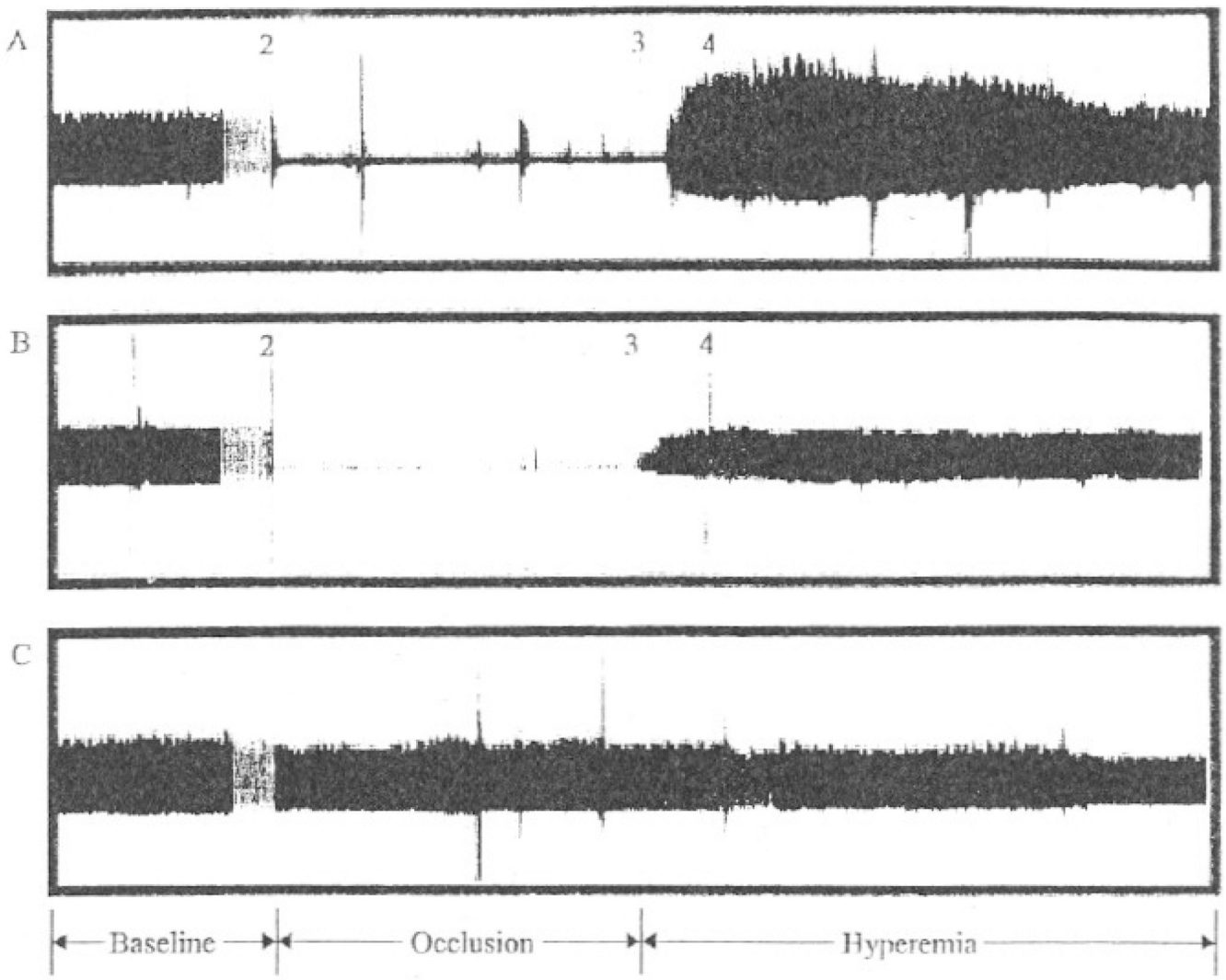

FIG. 1.

Pulse amplitude recorded in the index finger with the Endo-PAT 2000 device (Itamar Medical, Ltd.) before, during, and after cuff occlusion of the arm, as described in the text. (A) The response from a healthy individual with no risk factor. (B) The very blunted response in an individual with coronary artery disease. (C) The response in the contralateral finger not subject to cuff occlusion, which demonstrates that the signal remains stable over time. The PAT ratio is calculated at baseline and between 1 and 2 min after cuff release. Reproduced, with permission, from Kuvin *et al.* (2003).

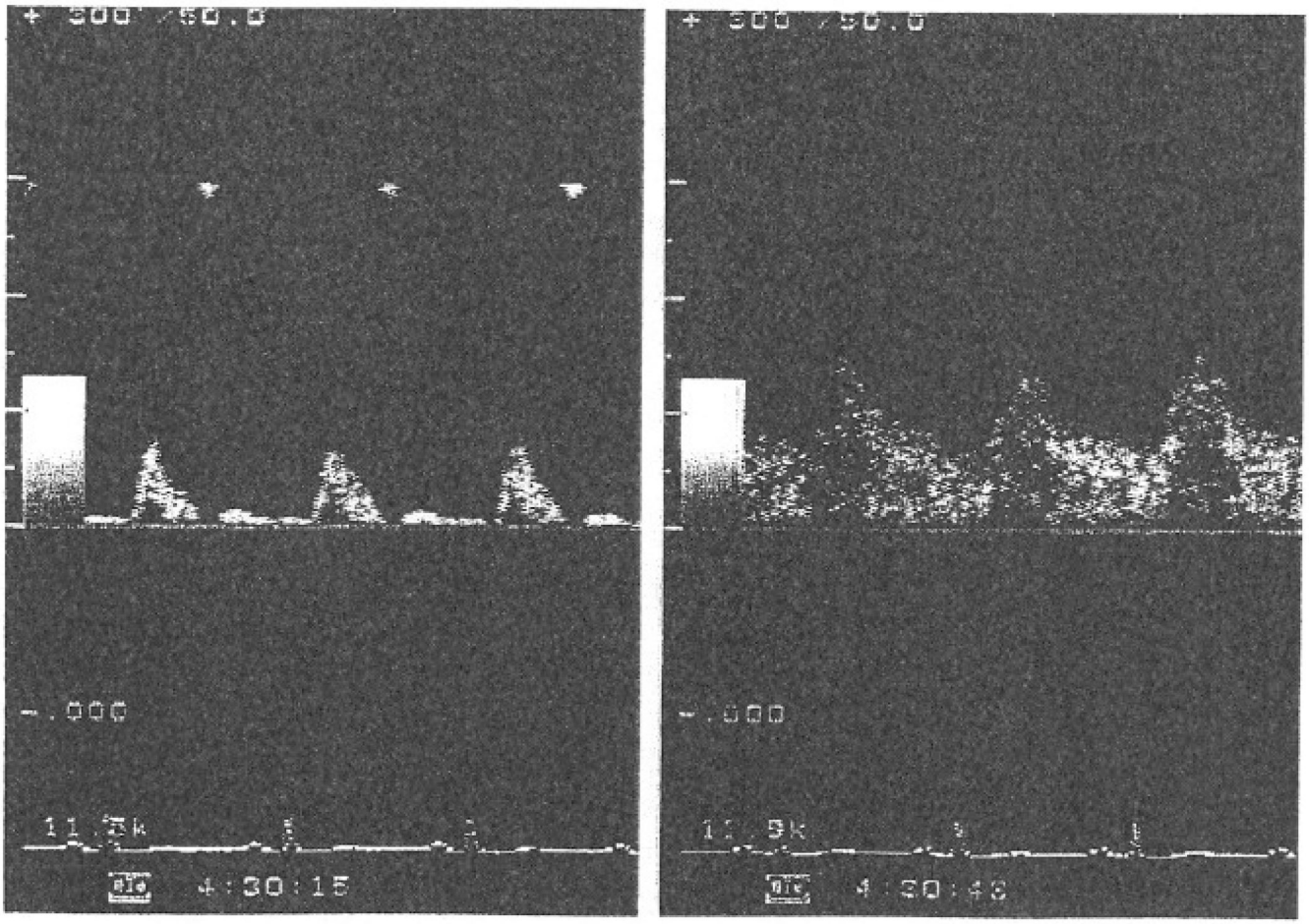

FIG. 2.
Representative Doppler recordings from the brachial artery at baseline (left) and immediately after cuff release (right) reflecting hyperemic flow.

TABLE I

Noninvasive methods for study of NO-dependent vasodilation in human subjects

Method	Vascular bed	Stimulus for NO release	Advantages	Disadvantages
Vascular ultrasound Flow-mediated dilation (FMD)	Conduit brachial artery	Flow produced by reactive hyperemia	Well established Demonstrated prognostic Value Correlates with coronary circulation	Operator dependent Low signal/noise ratio Lack of standardized methodology
Pulse amplitude tonometry (PAT)	Small arteries in the fingertip	Flow produced by reactive hyperemia	Automatic Rapid results Correlates with coronary circulation	Clinical relevance not established Minimal information about response to interventions
Venous occlusion Plethysmography	Forearm resistance vessels	Flow produced by reactive hyperemia	Stronger correlation with risk factors than FMD Can be measured by several different techniques	Low reproducibility Operator dependent
Arterial compliance Pulse wave analysis	Central aorta or brachial artery	None	More reproducible and stable overtime Low operator dependence	Only partially NO dependent Relates to arterial structure and vascular tone
Transthoracic Doppler	Left anterior descending coronary artery	Adenosine or adenosine triphosphate	More clinically relevant circulation	Only partially NO dependent Highly operator dependent Lack of standardized methodology

TABLE II

Mean values with 95% confidence intervals for brachial artery flow[a]

Age (y)	Sample size	Hyperemia volume flow ratio	Baseline flow velocity (cm/s)	Hypermia flow velocity (cm/s)
<30	n = 173	8.0 (6.0–10.1)	12.0 (11.1–13.0)	79.5 (75.2–83.7)
30–39	n = 125	7.3 (6.6–8.0)	11.4 (10.3–12.5)	78.0 (72.2–83.9)
40–49	n = 121	6.9 (6.1–7.6)	11.9 (10.7–13.0)	77.6 (72.0–82.3)
50–59	n = 56	6.7 (5.6–7.7)	11.4 (9.9–13.0)	74.1 (64.6–83.4)
≥50	n = 37	5.1 (3.3–6.9)	12.2 (9.9–14.6)	63.5 (50.1–76.2)

[a]Displayed are mean values and 95% confidence intervals according to age. Results Shown are for subjects without clinical history of coronary artery disease, peripheral vascular disease, diabetes mellitus, or hypertension.