The arterial wall has 3 layers (ie, the intima, including the endothelium, the media, and the adventitia); each of these layers has individual roles in systemic circulation. The vascular endothelium regulates the vascular tone, hemostasis and/or vascular permeability, and the media is the major determinant of arterial elasticity, which regulates the conduit function (delivery of blood to tissues) and cushioning effect (for generation of continuous blood flow). Failure of these functions results in organ/vascular damage. Several non-invasive methods are currently used to assess vascular dysfunction, including measurement of flow-mediated vasodilatation of the brachial artery induced by reactive hyperemia (FMD), pulse wave velocity (PWV), the augmentation index (AI), and central blood pressure. Endothelial dysfunction, which is assessed by FMD, contributes to the initiation/progression of atherosclerosis. Increased arterial stiffness, which is assessed by the PWV and/or AI, causes increased cardiac afterload, impaired coronary arterial blood supply, atherogenesis and/or microvascular damage. The combination of risk stratification by assessment of conventional risk factors for cardiovascular disease (CVD) with not only a morphological assessment of vascular damage, such as carotid ultrasound examination, but also vascular function tests, may be a useful strategy for the management of CVD and its related risk factors. (Circ J 2010; 74: 24–33)

**Key Words:** Arterial stiffness; Central blood pressure; Endothelial function; Pulse wave velocity; Wave reflection

The clustering of conventional risk factors for cardiovascular disease (CVD) is associated with exaggerated vascular damage and poorer outcomes. Therefore, clinical medicine and public health policies place significant emphasis on the modification of conventional risk factors and lifestyle behaviors to reduce the epidemic of CVD. Although the population-attributable risk of major vascular risk factors is substantial, it is often difficult to distinguish those individuals with a moderate baseline risk who might benefit from aggressive risk reduction strategies. Furthermore, some studies have reported that the traditional cardiovascular risk factors account for “only 50%” of those who go on to develop coronary heart disease. Therefore, additional tests to assist in the prediction of the cardiovascular risk in these individuals may be warranted.

Ultrasound examination of the carotid artery is increasingly being used as a surrogate marker of atherosclerosis and is a strong predictor of future vascular events. However, evaluation of the regression of subclinical atherosclerosis by carotid arterial ultrasound examination following treatment of risk factors takes time (ie, more than 1 year is required in most cases to confirm such regression), and therefore more sensitive markers to assess the effect of treatment of risk factors on vascular damage are proposed.

Risk factors for CVD cause not only structural, but also functional vascular damage. Several non-invasive methods are currently used to assess functional vascular damage, including measurement of flow-mediated vasodilatation (FMD) of the brachial artery induced by reactive hyperemia, pulse wave velocity (PWV), augmentation index (AI), central blood pressure (BP), etc and these vascular function tests have attracted attention as new tools for determining CVD risk. In this review, we describe the pathophysiology of vascular functions, the non-invasive methods of evaluating vascular functions and the clinical applicability of these tests for CVD risk stratification.

**Functions of Arteries**

The arterial wall has 3 layers (ie, the intima, including the endothelium, the media, and the adventitia) and each of these layers has a role in systemic circulation.

**Functions of the Endothelium**

Endothelium regulates vascular tone, hemostasis and/or vascular permeability. To exert these functions, endothelial cells biosynthesize several vasoactive substances, and of these, nitric oxide (NO) in particular has a pivotal role in protecting against the initiation/progression of atherosclerosis via its vasodilatory activity and its inhibitory activity against vascular smooth muscle cell growth, nuclear transcription of cell adhesion molecules, platelet aggregation and transcription of cell adhesion molecules, platelet aggregation and aggregation.
leukocyte adhesion to endothelial cells.\textsuperscript{16–19}

**Functions Related to the Vascular Medial Layer (Elasticity)**

The arterial tree can be simply classified into 3 compartments: elastic arteries, muscular arteries, and arterioles. Among the most important functions of the arterial tree are (1) delivery of blood to tissues and (2) its cushioning effect (to generate continuous blood flow). The elastic arteries have a high content of elastic fibers in the medial layer, and this elasticity plays a pivotal role in the 2 aforementioned functions.\textsuperscript{13,20,21} The elastic arteries have a dominant role in cushioning against and dampening the pressure oscillations that result from intermittent ventricular ejection (ie, the Windkessel effect) and transforming the pulsatile flow into a steady flow for supplying oxygen to the tissues.\textsuperscript{20} This elasticity has beneficial effects on the cardiovascular system, such as facilitating organ blood supply during diastole, especially in the heart, reduction of cardiac afterload, and/or the protection of the arterial wall and microvasculature from the mechanical stresses generated by cardiac contractions.\textsuperscript{13,20,21}

### Non-Invasive Methods of Assessing Vascular Functions

**Endothelial Functions**

Apart from biochemical markers,\textsuperscript{22} the assessment of endothelium-dependent vasodilatation is widely used to evaluate endothelial function.\textsuperscript{8,22} Measurement of the brachial artery diameter before and after an increase in shear stress induced by reactive hyperemia (ie, FMD) is most frequently used in the clinical setting\textsuperscript{8,9,22,23} (Figure 1). The increased shear stress induced by reactive hyperemia activates endothelial NO synthase (eNOS) and increases NO production in the endothelium. Thus, FMD is a non-invasive marker of local NO bioavailability in the endothelium, which is an important factor in protecting against initiation/progression of atherosclerosis.\textsuperscript{24,25} FMD measurement requires appropriate training for technical validation and good reproducibility\textsuperscript{22,23} and semi-automated equipment resolves such technical limitations.\textsuperscript{26} Non-invasive assessment of the changes in forearm blood flow by venous occlusion plethysmography has been used as a marker of vascular reactivity,\textsuperscript{27} but prostaglandins and endothelial-derived hyperpolarizing factor, in addition to NO, may also contribute to increased forearm blood flow induced by reactive hyperemia.\textsuperscript{27,28}

Sublingual nitroglycerin administration also produces endothelium-independent brachial arterial vasodilatation (NMD),\textsuperscript{8,10} which reflects vascular smooth muscle cell function. Although several studies have demonstrated impaired smooth muscle cell function in subjects with risk factors for CVD,\textsuperscript{29,30} the significance of NMD as a useful marker for CVD risk stratification is not yet fully established.

**Assessment of Arterial Elasticity**

**PWV** PWV reflects segmental arterial elasticity. Contraction of the left ventricle generates a pulse wave that is propagated throughout the arterial tree. PWV is calculated as the distance traveled by the pulse wave divided by the time taken to travel the distance.\textsuperscript{12,31} Increased arterial stiffness is associated with an increased propagation speed of the pulse wave in the artery (Figure 2). PWV can be measured in any arterial segment between 2 pulse-wave palpable regions.\textsuperscript{31} Assessment of central arterial stiffness rather than peripheral arterial stiffness is more relevant to CVD risk stratifica-
**Figure 2.** Schema of propagation of the incident pulse wave and reflected pulse wave in the arterial tree.

**Figure 3.** Schema of measurement of the carotid–femoral pulse wave velocity (PWV) and brachial–ankle PWV.
Radial AI and Central BP

Central BP as estimated from the carotid AI has been shown to be reliable, but validation of the central BP as estimated from the radial AI is still under debate. Even so, measurement of the radial AI and central BP as estimated from the radial AI are simple enough for application to a large number of study subjects and also in routine practice.

Conventionally, increased stiffness in the arterial tree is thought to increase the traveling speed of the incident pulse wave and to shift the reflection point to a proximal site in the arterial tree (Figure 2). Thus, macrovascular dysfunction is related to the former phenomenon and microvascular dysfunction to the latter phenomenon. However, recent studies suggest that macrovascular dysfunction causes impedance mismatching between the macro- and microvasculature and that this mismatch shifts the reflection point distally in the arterial tree. Thus, the interaction between the effects of macrovascular function and microvascular function on pressure wave reflection has not yet been fully clarified.

Local Arterial Distensibility in the Carotid Artery or Aorta

Using ultrasound examination or magnetic resonance imaging (MRI), the maximum and minimum diameters of arteries in systole and diastole are obtained with simultaneous recording of the BP. Thus, the local arterial distensibility can be calculated, and this parameter has been used in clinical research. However, because BP differs in the different regions of the arterial tree, the BP at the site of examination of local arterial distensibility needs to be obtained for its calculation. In the measurement of carotid arterial distensibility using ultrasound, morphological assessment of carotid arterial damage can be conducted simultaneously, which is a methodological advantage.

Mechanisms of Vascular Dysfunction and Their Harmful Effects on the Cardiovascular System

Endothelial Dysfunction and Its Pathophysiological Link to Atherosclerosis and Arterial Stiffness

Endothelial dysfunction occurs in the early stage of atherosclerotic vascular damage, and NO bioavailability in the endothelium is impaired. An imbalance of NO production and/or increased NO inactivation underlies impaired NO bioavailability. eNOS activity and/or asymmetric dimethylarginine (ADMA) are reported to affect NO production. The conventional risk factors for CVD, such as aging, obesity, hypertension, smoking, dyslipidemia and/or diabetes mellitus inactivate eNOS. On the other hand, these conventional risk factors also activate nicotinamide-adenine dinucleotide phosphate (NADPH) activity, which increases NO inactivation.
vation via production of reactive oxygen species.\textsuperscript{52} Thus the accumulation of conventional risk factors for CVD exaggerates not only attenuation of NO production, but also increased NO inactivation. The impaired NO bioavailability contributes to the initiation/progression of atherosclerosis via attenuation of the aforementioned anti-atherogenic actions of NO, such as its inhibitory effect on vascular smooth muscle cell growth, nuclear transcription of cell adhesion molecules, platelet aggregation, and leukocyte adhesion to endothelial cells.\textsuperscript{16}

Endothelial dysfunction causes vascular inflammation and inflammation increases the production of cytokines and/or vasoactive substances (ie, angiotensin II and endothelin-1) in the vascular wall. In addition to impaired NO bioavailability, these inflammatory responses in the vascular wall are thought to stiffen the arteries both functionally and structurally.\textsuperscript{50}

**Increased Arterial Stiffness: A Risk Factor for Cardiovascular Events**

Functional/structural stiffening of the arteries occurs via several mechanisms.\textsuperscript{53–56} Increased arterial wall tension causes functional arterial stiffening. In addition to endothelial dysfunction, elevated BP, increased heart rate and/or sympathetic activation also increase functional arterial stiffness.\textsuperscript{20,53}

The mechanisms of stiffening of the vessel wall are not similar in all segments of the arterial tree because the constituents of the arterial wall vary along the longitudinal axis of the arterial tree (ie, elastic fibers are dominant in the elastic arteries, whereas collagen and smooth muscle cells are dominant in the muscular arteries).\textsuperscript{11,13,20,53} The arterial media is composed of smooth muscle cells and extracellular matrix (collagen and elastin). These constituents of the media and their interactions are major determinants of structural arterial stiffness.\textsuperscript{20,53,54} Conventional risk factors for CVD induce dysregulation of the balance between collagen and elastin, resulting in overproduction of abnormal collagens and diminished production of normal elastin. In addition, deposition of glycoproteins and proteoglycans on the vessel wall, cross-linkage of collagen molecules and the number of elastin/smooth muscle cell connections, which influence the extent of the elastin network anchorage to the smooth muscle cells, may also contribute to structural arterial stiffening.\textsuperscript{20,53–56}

The risk factors for CVD differ in their effects on structural arterial stiffening. Aging and hypertension cause thinning, splitting, fraying and fragmentation of elastic fibers. Vascular inflammation related to risk factors causes degradation of collagen and elastin, changes in the proteoglycan composition and hydration status, and medial calcification. Diabetes increases the accumulation of advanced glycation endproducts on collagen and elastin.\textsuperscript{54}

Increased arterial stiffness, especially of the larger arteries, is thought to act as a risk factor for cardiovascular events via several mechanisms (ie, increased cardiac afterload, impaired coronary blood flow, direct atherogenic actions and/or microvascular damage).\textsuperscript{11–13,21}

**A. Increased Cardiac Afterload**

Heart ejection into a stiffening artery increases the end-systolic pressure, resulting in cardiac hypertrophy and a greater energy requirement for cardiac contraction.\textsuperscript{53} The risk factors for CVD cause not only macrovascular dysfunction, but also microvascular dysfunction.\textsuperscript{57–59} Increased oxidative stress and/or inflammation related to these risk factors causes arteriolar remodeling and microvascular rarefaction.\textsuperscript{59} Increased stiffness of the large arteries and these microvascular alterations hasten the return of the pulse wave reflection from the periphery,\textsuperscript{13,21} which in turn augments the ventricular pressure during systole.\textsuperscript{13} (Figure 5).

**B. Impaired Coronary Blood Flow**

The cushioning effect of elastic arteries involves their expansion during systole to store some of the blood ejected from the heart, and delivery of this stored blood to the tissues in diastole.\textsuperscript{13,21,60} Coronary arteries are mostly perfused during diastole, and therefore the amount of this stored blood is an important determinant of the coronary blood flow.\textsuperscript{13,21} Furthermore, in a healthy arterial tree, the speed of propagation of the pulse wave (incident (heart to periphery) and reflected (periphery to the heart)) is not very high, so the reflected wave returns to the central aorta during diastole. This phenomenon elevates the diastolic BP and contributes to maintenance of the coronary blood flow.\textsuperscript{13}

With increased arterial stiffness, the amount of stored
blood in the large arteries during systole is decreased and the elevation of the diastolic pressure by the reflected pulse wave is reduced, because the reflected pulse wave returns during systole as a result of the increase in the pulse wave propagation speed (Figure 5); coronary blood flow is impaired.13,21,60

C. Arterial Remodeling and Atherogenic Actions The arterial wall is exposed to 2 mechanical stresses: circumferential stress (stretch) and shear stress. Increased arterial stiffness is associated with an increase of both stresses because of the increased pressure and speed of blood flow in the arterial lumen.59 These stresses trigger signaling cascades in the arterial wall, such as tyrosine kinase, phosphatidylinositol-3-kinase, protein kinase C, the epithelial sodium channel, and/or NADPH oxidase, contributing to vascular growth.61 In addition, local arterial stiffness may affect plaque vulnerability.62

D. End-Organ Damage Attenuation of the cushioning effect of elastic arteries amplifies the pressure pulsatility and increases the transmission of pulsatile energy to the peripheral microcirculation (Figure 6). In particular, in high-blood-flow organs, such as the brain and kidney, pressure pulsatility penetrates further into the microcirculation, which causes brain and kidney damage.63

Evidence of Vascular Function Tests as Markers of CVD Outcomes

FMD and Prognosis Several studies have demonstrated that FMD is a predictor of the clinical outcome in patients with CVD.64–66 which suggests that FMD may reflect plaque destabilization via local inflammation and prothrombotic tendency.67 However, the samples in those studies were relatively small. In contrast, in studies of larger numbers of subjects, although FMD provided incremental prognostic information in 2,264 post-menopausal women,68 the usefulness of the parameter as a prognostic indicator was relatively limited in 2,792 elderly subjects69 and 842 multi-ethnic cohorts.70 Thus, further studies of a larger number of subjects, especially low-risk subjects,71 are needed to confirm the usefulness of FMD as a predictor of CVD outcomes.

PWV and Prognosis In large numbers of healthy subjects or hypertensive subjects, the carotid–femoral PWV has been demonstrated to be a predictor of future cardiovascular events.72–75 whereas the usefulness of the brachial–ankle PWV as a marker of prognosis has been demonstrated in some limited subjects, such as those with acute coronary syndromes, end-stage renal disease or heart failure.76–78 Further accumulation of data to confirm the usefulness of the brachial–ankle PWV as a predictor of the prognosis is required.

Aging, BP and other atherogenic factors cause vascular damage that produces structural stiffening of the arteries.12,20,53 On the other hand, increased BP increases arterial wall tension, causing functional stiffening of the arteries. Traditionally, PWV has been used as a marker of atherosclerotic vascular damage, but from this point of view, BP is a confounding variable, because structural arterial stiffening may also be related to atherosclerotic vascular damage. Recently, however, several other methods (ie, ultrasound examination, computed tomography and/or MRI) have been used to assess the severity of atherosclerosis with greater precision. Thus, at present, PWV is considered as a marker of the risk of CVD, rather than of atherosclerotic vascular damage.11,12 The aforementioned mechanisms are thought to contribute to the increased risk of CVD associated with arterial stiffening. From this point of view, BP may be a significant factor.

PWV is a marker of segmental arterial stiffening, including the influence of BP. The stiffness index, β, is a marker of

![Figure 6. Cushioning effect of large arteries on the pulsatile energy generated by the heart.](image-url)
arterial stiffness with minimal influence of BP; however, this parameter is a marker of regional and “not segmental” arterial stiffness.12,47,48 Beta can be measured at the brachial, femoral or carotid artery, or at the aorta, after adjustment for BP at the same site. In the arterial tree, BP differs in different regions (usually, the level is low at proximal sites and high at distal sites).13,14 Thus, it is important to understand that \( \beta \) cannot be used to obtain segmental arterial stiffness like the PWV, because BP differs between the proximal and distal sites. Further studies are needed to clarify whether PWV or \( \beta \) is the more useful marker of the risk of CVD.

**AI, Central BP and Prognosis**

Some studies conducted on relatively small numbers of subjects have shown that the non-invasively estimated AI and/or central BP predicts prognosis in subjects with end-stage renal disease or coronary heart disease, American Indians and hypertensive subjects.79–82 However, conflicting results have also been reported.83,84 Age, sex and heart rate are important determinants of the AI,46,85 so their influence on the significance of AI and central BP as markers of prognosis should be clarified.

Recently, the CAFÉ study suggested that reduction of central BP is related to improved prognosis.86 Thus, the AI and central BP may be promising as markers of the effect of interventions for risk factors of CVD outcomes.

**Prediction of End-Organ Damage**

FMD is a predictor of the progression of structural carotid artery disease,67 and the PWV is reported as a predictor of new onset of hypertension.87,88 Thus, vascular function tests have the potential to predict progression of vascular damage and end-organ damage. Furthermore, vascular function tests may be useful tools for assessing the preventative effects of modulation of risk factors for CVD.

**Perspectives and the Next Step**

Although morphological and functional assessments of vascular damage are markers of cardiovascular outcomes, the former may reflect the current severity of vascular damage and the latter may provide the risk of progression of organ/vascular damage. Thus, vascular function tests may provide further information for the management of CVD and its risk factors, in addition to morphological assessments. The combination of these 2 approaches may be useful for risk stratification (Figure 7). However, because the number of subjects requiring cardiovascular risk stratification is extremely large, all of the methods of morphological and functional assessment of vascular damage cannot be used in all of the subjects. Thus, the establishment of efficient strategies for risk stratification using new serological markers,89,90 the morphological markers of vascular damage and vascular function tests (ie, selecting the best one, the best combination, or all), in addition to assessment of the risk factors for CVD, is needed.

According to the published evidence, we make some comments concerning the aforementioned strategies.

1. In low risk subjects; the usefulness of carotid ultrasound examination for cardiovascular risk stratification might be limited.91 In low-risk subjects with high normal BP, the PWV may be a predictor of the risk of progression to hypertension. In low-risk subjects undergoing lifestyle modifications, FMD may be a sensitive marker of the effects of such modification on vascular damage, and improvement of this parameter may improve the subject’s motivation to maintain a beneficial lifestyle.

2. In high-risk subjects, the combination of morphological and functional assessments of vascular damage might be useful for risk stratification, because both assessments reflect different facets of the pathophysiological abnormalities underlying vascular damage.
3. In subjects with subclinical atherosclerosis detected by morphological assessment, the addition of functional assessments of vascular damage is proposed.

4. FMD, the PWV and the AI reflect different facets of the pathophysiological abnormalities underlying functional vascular damage. Witte et al reported from their meta-analysis that FMD is related to the principal cardiovascular risk factors only in low-risk populations.39 McEniery et al proposed that the AI might be a more sensitive marker of arterial stiffening and cardiovascular risk in younger individuals, whereas the aortic PWV is likely to be a better measure in older individuals.34 Thus, further study is needed to compare the usefulness of each of the vascular function tests among different cardiovascular risk profiles.

In the management of the risk factors for CVD, although lifestyle modification and/or treatment of these risk factors improves functional and/or structural vascular damage,5,10,12,14,20,22,30-32 no studies have confirmed the existence of a relation between such regression and improvement of the cardiovascular outcomes. This confirmation is needed to establish the applicability of functional and structural assessments of vascular damage as markers of the effect of treatment of CVD and its risk factors on outcomes.

References


73. Williams B, Lacy PS, Cruickshank JK, Collier D, Hughes AD,


