Cardiology Research and Practice

# The Arterial Pulse: Vascular Biology, Vascular Function Testing, and Therapies

Guest Editors: A. Maziar Zafari, Arshed A. Quyyumi, Julian P. J. Halcox, and Sanjay Rajagopalan

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*Editorial*

# The Arterial Pulse: Vascular Biology, Vascular Function Testing, and Therapies

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In this special issue on The Arterial Pulse: Vascular Biology, Vascular Function, and Therapies, we have compiled five reviews, a clinical trial and a translational pilot study for the readership of Cardiology Research and Practice.

Techniques and approaches for modern vascular function testing have evolved over time from invasive methods restricted to smaller studies in the research laboratory to more standardized noninvasive methods, which are suitable for use in large prospective cohort studies and clinical trials. E. A. Ellins and J. P. J. Halcox describe currently available methods for the assessment of endothelial function and their potential application in cardiovascular research and clinical practice. In the second contribution E. Patvardhan and co-authors present a clinical trial investigating the relationship of the augmentation index (AIx) obtained from pressure waveforms via arterial applanation tonometry and cardiovascular risk factors and coronary artery disease (CAD). The authors conclude that AIx may be a useful measure of assessing overall risk for coronary heart disease. J. Steppan and colleagues review the effects of age-associated increase in vascular stiffness on systolic blood pressure, pulse pressure, AIx, and cardiac workload in the third paper. In this paper they describe evidence for the use of pulse wave velocity testing to measure vascular stiffness as an index of vascular health and as a predictor of adverse cardiovascular outcomes. In the fourth paper, M. Weber and colleagues present data from a pilot study about the potential diagnostic role of microRNAs (miRNAs) in blood samples of patients with angiographically documented CAD and healthy controls. The authors show that a distinct miRNA expression profile discriminates patients with CAD from healthy controls, which in turn is altered by vasoactive medications such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. In the fifth paper, C. R. Martens and D. G. Edwards review the current literature pertaining to the potential mechanisms of peripheral vascular dysfunction in chronic kidney disease and propose possible targets for treatment.

Vascular endothelial dysfunction is associated with a reduction in nitric oxide (NO) bioavailability, an increase in vasoconstrictors, including superoxide anions and endothelin-1 in parallel with a potential compensatory increase in other mediators of vasodilatation. This non-NO, non-prostaglandin-mediated endothelium-dependent vasodilatation has been partly attributed to endothelium-derived hyperpolarizing factors (EDHFs), the topic of the comprehensive review by M. A. Ozkor and A. A. Quyyumi who describe the role of endothelial hyperpolarization in human circulatory physiology. Finally, N. Ghasemzadeh and A. M. Zafari provide a historical review about the evolution of our knowledge of the arterial pulse from ancient times to the modern era. The authors describe the revolutionary scientific concepts and the technological innovations that provided the foundation of our current understanding of

the arterial pulse as a wave that can be accurately measured with various noninvasive techniques in standardized fashion suitable for clinical patient-oriented research as well as clinical practice.

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*Review Article*

# Where Are We Heading with Noninvasive Clinical Vascular Physiology? Why and How Should We Assess Endothelial Function?

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There are several invasive and noninvasive methods available to the clinical researcher for the assessment of endothelial function. The first investigations in humans involved invasive pharmacological vascular function testing, which have been used to gain a detailed understanding of the mechanisms involved in the pathogenesis of endothelial dysfunction and atherosclerosis as well as novel targets for intervention. Techniques for endothelial function testing have evolved over time from these invasive methods, which, by their nature, are restricted to small studies in the research laboratory, to more standardized noninvasive methods, which are suitable for use in large prospective cohort studies and clinical trials. This paper describes currently available methods for assessment of endothelial function and their potential application in cardiovascular research and clinical practice.

## 1. Introduction

The single-cell monolayer that lines the vascular system, the endothelium, is responsible for homeostasis of the blood vessels. It releases a number of factors that play a role in the regulation of vascular tone, the inhibition of platelet aggregation, and the adhesion of leukocyte. Disturbances in these processes cause endothelial activation and dysfunction, characterized by a decrease in the availability of the endothelium-derived vasodilator nitric oxide (NO) and the activation of vasoconstrictors such as endothelin-1 and angiotensin II. In combination with the expression of prothrombotic, proinflammatory, and adhesion molecules, this initiates an environment favourable to the development of atherosclerotic lesions. These lesions eventually develop into plaques, which can gradually progress to luminal obstruction and ischemia, or destabilize and rupture resulting in acute events such as myocardial infarction and stroke. Endothelial dysfunction is the earliest clinically identifiable event in the process of cardiovascular disease.

## 2. Rationale for Testing Endothelial Function

Furchgott and Zawadzki were the first to demonstrate that endothelial cells were required for smooth muscle relaxation to occur in response to acetylcholine administration in rabbit aorta [1]. Nitric oxide was later identified as the substance responsible for this relaxation [2]. Based on this and other works, Ludmer et al. were the first to show that acetylcholine caused vasoconstriction in atherosclerotic arteries and vasodilatation in healthy vessels in the human coronary circulation during cardiac catheterization [3]. Assessing changes in forearm blood flow in response to pharmacological probes allows a more detailed exploration of resistance vessel function, but these invasive studies are only appropriate for use in small study cohorts. In 1992, Celermajer et al. developed a noninvasive method for assessing endothelial function in the conduit arteries of the peripheral circulation [4]. This technique uses a period of forearm ischemia followed by reactive hyperemia to increase brachial arterial blood flow and, consequently, shear stress. This induces endothelial release of NO which mediates

smooth muscle relaxation and vasodilatation of the brachial artery [5]. Since then other techniques have been developed that either employ reactive hyperemia or administration of drugs to stimulate the endothelium. Endothelial vasodilator function in the peripheral circulation has been shown to be related to coronary endothelial function and cardiovascular risk factors such as dyslipidemia, smoking, and diabetes and to be predictive of cardiovascular events [6–10].

## 3. Methods for Assessing Endothelial Function

Several invasive and noninvasive methods have been developed for the assessment of endothelial function. No single method is the perfect test, and a combination may be required for a comprehensive evaluation of vascular endothelial biology.

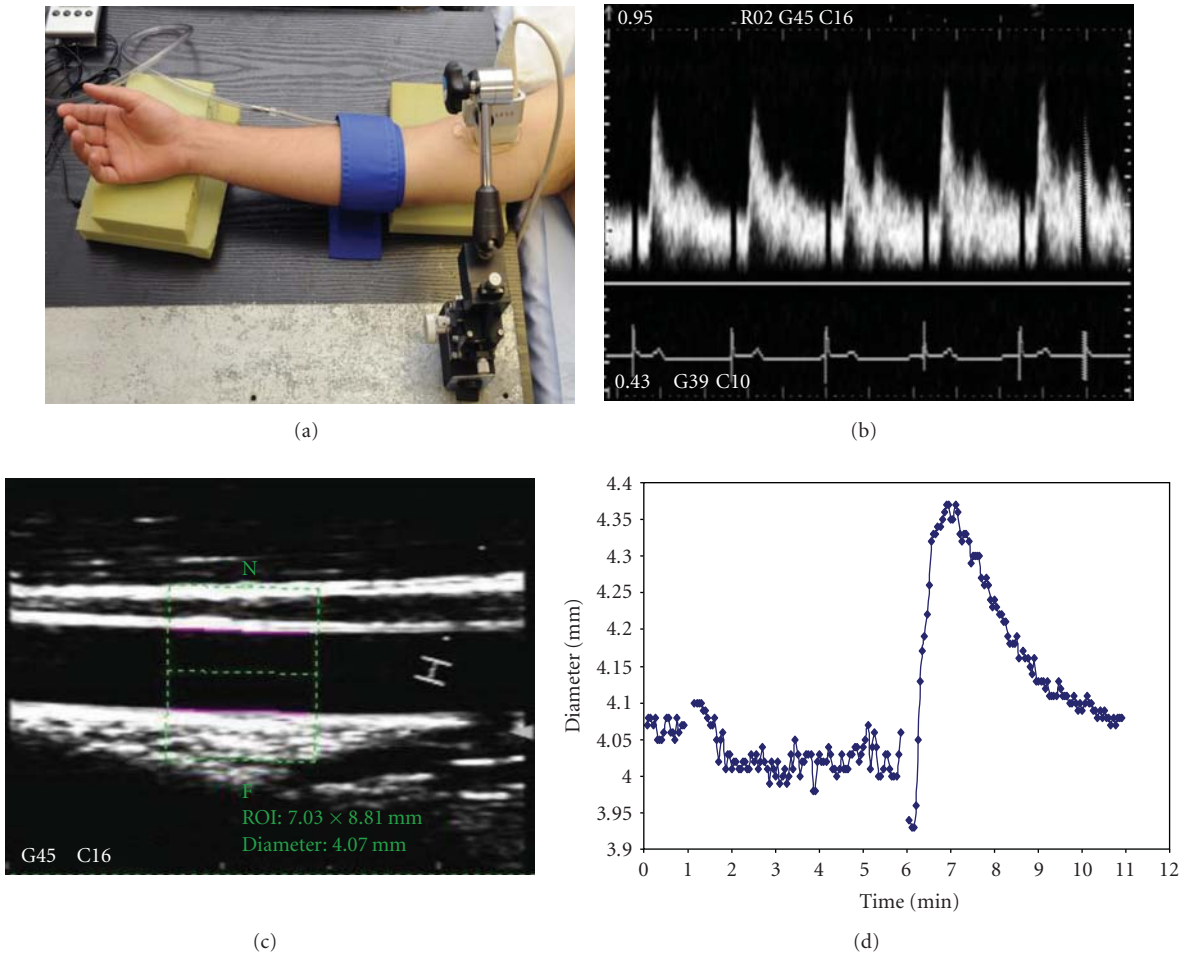
*3.1. Invasive Assessment of Endothelial Function.* The original clinical investigations of endothelial vasomotor function assessed the coronary circulation. Changes in the epicardial and microvascular responses to endothelium-dependent pharmacological agents are measured during cardiac catheterization using quantitative coronary angiography and the Doppler flow-wire techniques. Preserved epicardial coronary endothelial function is characterized by vasodilatation in response to acetylcholine. Constriction of the vessel is indicative of the smooth muscle response to direct muscarinic receptor stimulation overwhelming the absent or depressed dilation that follows from reduced bioavailability of endothelial NO [11]. the use of this technique is realistically restricted to individuals in the more advanced stages of arterial disease as it should only be carried out in those with clinical indications for cardiac catheterization. However, coronary vascular function testing has provided important insights into the effects of atherosclerosis and its risk factors on coronary regulatory physiology and risk stratification as well as demonstrated the potential reversibility of endothelial dysfunction in response to treatments such as statins and ACE-inhibitors [12–14].

Forearm resistance vessels can be studied using venous occlusion plethysmography to assess changes in forearm blood flow (FBF) in response to pharmacological agents. This technique provides its own control by using the contralateral arm, permitting adjustments to be made for systemic influences that affect basal flow and blood pressure in the noninfused arm. The majority of studies measure percentage differences in FBF and vascular resistance between experimental and control arms following administration of endothelium-dependent and endothelium-independent agonists. Evaluation of the contribution of NO to vasomotor regulation can be made using eNOS antagonists such as L-NMMA. This technique can be used in healthy controls as well as patients, allowing the study of the endothelium from early in the disease process. It also allows other vasomotor pathways in addition to NO to be evaluated. However, it remains an invasive technique which limits its repeatability and also restricts its use to small studies. Also, the clinical relevance to atherosclerosis has been questioned, as microvascular pathophysiology may not necessarily reflect changes in the conduit arteries in which the plaques develop.

*3.2. Noninvasive Methods of Assessment.* The current gold standard technique for noninvasive assessment of the endothelium is flow-mediated dilatation (FMD) using ultrasound. This method is based on the vasodilatation initiated by the reactive increase in blood flow in the brachial artery (or other conduit arteries) following a 5-minute period of forearm ischemia mediated by suprasystolic inflation of a blood pressure cuff on the arm. The resultant hyperemia following cuff release causes an increase in local shear stress in the vessel which stimulates the endothelium to generate and release NO, which activates guanylyl cyclase to produce cyclic GMP in vascular smooth muscle which causes relaxation and dilatation of the artery [5, 15]. The changes in blood flow and vessel diameter can be assessed by imaging the brachial artery and measuring blood flow with high-resolution 2D ultrasound and Doppler interrogation (Figure 1). It is also important to assess endothelium-independent vasodilatation for comparison, which reflects smooth muscle function, using the NO donor glyceryl trinitrate (GTN), usually administered sublingually.

A technically demanding technique and initially expensive to set up is FMD which is normally used in the research laboratory where it has been demonstrated to have good reproducibility [16]. However, it can also be used with care in large epidemiological studies and has an expanding role in clinical trials [17–20]. Some differences in technique and controversies remain regarding the most robust methodology for FMD. Both cuff position and duration of the occlusion period can affect the reactive hyperemic stimulus. If the occlusion cuff is positioned above the ultrasound probe, making the whole arm ischemic including the measured arterial segment, a larger dilatation of the vessel is seen than when the cuff is placed on the forearm distal to the study segment [15, 21]. A cuff occlusion of 15 minutes also causes a larger hyperemic response than of a 5-minute period [22]. Thus, both proximal cuff positioning and longer occlusion periods may not specifically represent NO-mediated endothelium-dependent vascular function [15, 23]. In contrast, a more distal positioning of the cuff and using a 5-minute occlusion period have been demonstrated to induce NO-mediated vasodilatation, although there is still some debate over this matter [15, 22, 24–26]. Another area of current interest is how to interpret and adjust for the reactive hyperemic stimulus. Previously, only the maximal reactive hyperemic response was typically reported (if at all), but more recently it has been recommended that the impact of the change in shear stress, represented as shear rate, should be considered over the entire period of reactive hyperemia using the integral of shear rate over time (AUC) [27]. Efforts have been made to reduce the variability in the methodology, which can limit comparison of the results from different FMD studies [28–31].

Other methods of assessing peripheral vasomotor function involve the administration of agents such as the $\beta 2$ adrenergic receptor agonist salbutamol, which can be




(a) (b) (c) (d)

Figure 1: Setup and representative data for ultrasound evaluation of brachial FMD. (a) A sterotatic clamp with micrometer adjustment is used to hold the ultrasound probe. The occluding cuff is positioned just below the elbow. (b) Doppler trace showing flow velocity profile during early reactive hyperemia. (c) B-mode image of brachial artery demonstrating selection of region of interest and edge-detection analysis with Brachial analyzer software package. (d) Brachial artery diameter profile during FMD protocol as measured using brachial analyzer the software package (0-1 minute baseline, 1–6 minutes cuff up, 6–11 minutes response to reactive hyperemia (FMD)).

administered via an inhaler or IV infusion and does not affect blood pressure at standard clinical doses [32–34]. Salbutamol stimulates the release of NO from the endothelium via vascular endothelial $\beta 2$ receptor activation. This reduces arterial tone and stiffness, which can be measured in the peripheral waveform with either pulse wave analysis (PWA) by applanation tonometry at the radial artery or pulse contour analysis (PCA) with digital photoplethysmography (Figure 2). Assessment of the changes on the central aortic waveforms can also be measured by using a transfer function which has been validated only in adults. Despite these techniques' relative simplicity and low cost, there are practical concerns regarding reproducibility compared with FMD especially if using PCA and in children [16].

Additionally, little correlation was observed between FMD and results with these techniques, implicating distinct pathophysiological influences at different levels of the vasculature requiring further evaluation [16].

Pulse amplitude tonometry (PAT) is another technically straightforward technique, which uses the same stimulus as FMD. The Endo-PAT system uses a fingertip probe to measures changes in arterial pulsatile volume. Recordings are made simultaneously in the right and left index fingers so providing an internal control. As a measure of reactive hyperemia, similarly provoked by a 5-minute period of forearm ischemia, the RH-PAT index is calculated as the ratio of the average amplitude of the PAT signal over a 1-minute time interval starting 1 min after cuff deflation, divided by the average amplitude of the PAT baseline (Figure 3). RH-PAT index values from the study arm are normalized to the control arm. Reproducibility has been demonstrated to be similar to that of FMD, and mechanistically the vasodilation is mediated at least in part by NO [35, 36]. However, it is not entirely NO dependent, and there is likely to be an important interaction with the autonomic nervous system that may confound interpretation of the results from a specific endothelial perspective. Another limitation of the technique

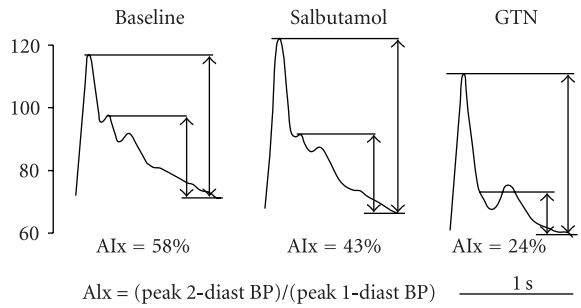



Figure 2: An example of the effect of salbutamol and glyceryl trinitrate (GTN) on the radial waveform in a single individual. The second systolic peak, obvious at baseline, is attenuated by salbutamol and almost completely abolished following GTN. Augmentation index (AIx), calculated as the ratio of the pulse pressure at the second systolic peak to that at the first systolic peak is used to quantify the changes in the waveform. BP = blood pressure. Printed with permission from [33].

is the inability to take into account the impact of autonomic influences on endothelium-independent response to systemic GTN due to lack of a simultaneous unexposed control arm.

Endothelial function can also be assessed using pulse wave velocity measurement. Arterial stiffness in the brachial artery reflects both arterial wall composition and smooth muscle tone. In response to a reactive hyperaemic stimulus, which increases shear stress and stimulates endothelial NO release, pulse wave velocity (PWV) slows due to the resultant drop in smooth muscle tone [37]. This simple technique, first developed by Naka et al. uses two cuffs, one placed at the wrist and one on the upper arm, to assess PWV in the arm; the reactive hyperaemic stimulus is induced by wrist cuff occlusion. An inverse relationship has been shown between PWV slowing and FMD using another method measuring carotid-radial PWV [38]. However these approaches still require further refinement and validation, but their relative simplicity is appealing for their potential application in larger-scale studies.

Increases in skin blood flow in the distal forearm following local thermal hyperemia, reactive hyperaemia, or iontophoretic application of endothelial agonists can be measured using the laser Doppler flowmetry: allowing assessment of cutaneous microvascular endothelial function. However, local changes in blood flow are only in part due to NO, with prostanoids also mediating some of the response [39, 40]. Reproducibility for this technique has been shown to be similar to that of FMD [41].

In conclusion FMD is the current gold standard and most used method for noninvasive assessment of endothelial vasomotor function. There are other less expensive and technically simpler methods emerging, most notably,s Endo-PAT, but questions remain regarding which pathways are being assessed by these techniques as responses may not correlate with FMD or are not fully attenuated by NO inhibition. Furthermore, some of these techniques may also be less sensitive and reproducible than FMD, thus necessitating larger study cohorts.

## 4. Clinical Application of Endothelial Function Testing

How Do We Apply the Methodology in Clinical Studies, What Can It Show Us and How Do the Results Enhance Our Understanding of Vascular Biology, Atherosclerosis and CVD?

*4.1. Role of Endothelial Function in Case-Control and Cohort Studies.* Endothelial function testing was initially a research laboratory-based technique in relatively small case-control, cross-sectional, and interventional studies. However, with the improvement in technology allowing smaller and cheaper equipment and the development of semiautomated analysis techniques, endothelial function testing is now being included in large prospective cohort studies. Indeed, Donald et al. have recently shown that FMD can be measured with high reproducibility in a large pediatric cohort of just under 8,000 participants, the Avon Longitudinal Study of Parents and Children (ALSPAC) [20].

One of the first cohort studies to measure endothelial function was the Cardiovascular Health study which assessed FMD in 2792 72–98-year olds between 1997 and 1998. This study was primarily investigating factors related to the onset and course of coronary heart disease and stroke, but also evaluated the prognostic ability of FMD to predict cardiovascular events in older adults [9]. Another cohort study, the Firefighters and Their Endothelium (FATE), was intentionally set up to assess the relationship between endothelial function, emerging risk factors, and atherosclerotic vascular disease in 1600 middle-aged fire fighters [42]. Other established longitudinal studies have also incorporated endothelial function testing into their screening visits. Between them, these cohorts cover a wide range of ages and exposures from prenatal and early life (ALSPAC) through later childhood into adulthood (Young Finns' and Framingham's offspring studies) [20, 43, 44]. From a practical perspective, it may not always be appropriate or logistically possible to administer GTN to study endothelium-independent vasodilatation within these studies, which can limit the specificity of the observations.

*4.2. Insights and Opportunities from Cohort Studies.* Cardiovascular risk factors have long been associated with diminished endothelial function, confirmed more recently in the larger cohort studies [45, 46]. In those with impaired FMD, risk factor burden is associated with increased intima-media thickness, an important subclinical marker of atherosclerosis [47]. Not all associations, however, have been consistently replicated in cohort studies, for example, the relationship between attenuated endothelial function and elevated CRP found in small cross-sectional studies [48–51]. However, FMD is diminished in children with acute and recent minor infections [52].

The prospective nature of these studies allows researchers to look at the potential impact of earlier risk factor exposure on contemporary endothelial function. For example, childhood blood pressure was demonstrated to be predictive

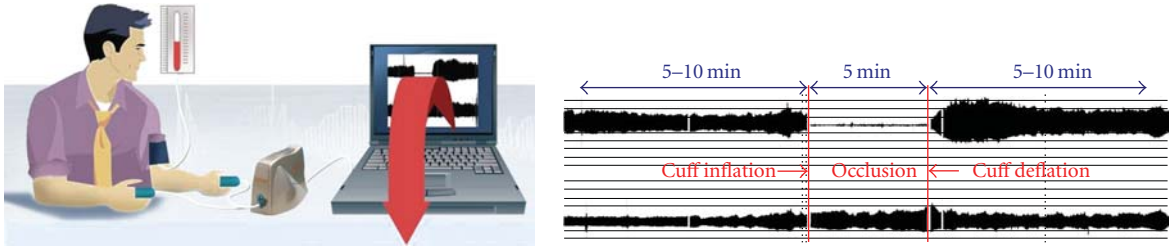



Figure 3: Diagram of the Endo-PAT set-up (a). (b) Representative traces from both the study and control arms showing the changes on the pulse amplitude after a 5-minute period of occlusion (printed with permission of Itamar Medical).

of endothelial dysfunction independent of presence and levels of other risk factors in adult life [53]. Associations found in cross-sectional analyses can also be followed up prospectively in the same cohort. For example, asymmetrical dimethylarginine (ADMA) was inversely correlated with FMD and subsequently found to predict FMD six years later [54, 55].

The prognostic ability of endothelial function testing can also be examined in prospective cohorts. Endothelium-dependent dilatation has been consistently shown to be significantly associated with progression of subclinical disease and incident clinical cardiovascular events in several studies [9, 56–58]. Although this association suggests a causally important influence on the pathogenesis of arterial disease, this alone is insufficient evidence to justify the use of endothelial function testing in routine clinical practice and data suggesting incremental predictive value, ideally with regard to improved ability of the results of such a test to reclassify the risk of an individual. Yeboah et al. demonstrated an overall 29% correct reclassification of subjects into low-, medium-, and high-risk groups with the addition of FMD to the Framingham Risk Score (FRS) in comparison to classification by FRS alone [59]. However, a more recent study found that only forearm resistance vessel endothelial function, and not FMD, was associated with 5-year risk of cardiovascular events [60]. It is important to note that in the latter study, the coefficient of variation for FMD was very high (29%) which may have limited the ability of the researchers to detect modest but important associations. This highlights very important practical concerns regarding the suitability of endothelial function testing for use in routine practice. The technical complexity of clinical endothelial function testing protocols together with the inherent physiological variability of endothelium-dependent vasodilatation under nonstandardized conditions means that such approaches are less practically suited for wider routine clinical use outside of the carefully controlled environment of a specialist vascular laboratory and currently remain predominantly in the research domain.

FDA approval has been granted for the use of the EndoPAT device for noninvasive detection of patients with coronary endothelial dysfunction following a small study of 94 subjects with chest pain who underwent invasive coronary angiography and endothelial function testing [61]. In this cohort, an EndoPAT index threshold of 1.67 provided a sensitivity of 82% and a specificity of 77% for coronary endothelial dysfunction which may help guide the management of challenging patients with chest pain and angiographically unobstructed coronary arteries but cannot justify wider clinical application of this technology at present.

*4.3. Role of Endothelial Function in Intervention Studies.* Endothelial function has increasingly become an attractive endpoint for clinical studies. Indeed it is possible to detect changes in endothelial function relatively quickly in comparison to other longer-term endpoints such as changes in the carotid or coronary wall and occurrence of clinical cardiovascular events. It can also provide the opportunity to evaluate potential "pleiotropic" influences of drugs. For example, statins have been shown to improve endothelial function prior to and independent of their effects on the lipid profile [62, 63]. Other pharmacological interventions such as renin-angiotensin system antagonists, calcium channel blockers, antiplatelet drugs, and angina treatments (ranolazine) have also been shown to have beneficial effects on endothelial function in addition to their primary actions [19, 64–67]. However, results from such studies are not always consistent in effect and results may vary between agents and according to the vascular bed studied. For example, in the BANFF study, FMD only improved after treatment with quinapril and not enalapril or losartan, whilst in another study cilazapril did not significantly improve endothelial function in the peripheral resistance vessels [19, 68]. Thus, it is important to account for the potential heterogeneity of endothelial biology when interpreting the results of clinical interventions. These issues have been reviewed in greater depth elsewhere [18, 69]. Nonpharmacological interventions such as Omega 3 supplements and exercise have also been shown to enhance endothelial function, which may in part account for their prognostic benefits [70–73]. Study methodology is of course critical in the setting of interventional studies. The study protocol and equipment must be standardized, and observers should be trained to a high level of quality and reproducibility [74]. Having proven itself as an endpoint in intervention studies FMD is now being used as an endpoint in drug development trials (e.g., Dal-Vessel) in order to provide valuable biological

insights at a relevant stage of the drug's clinical development [17]. Although a positive impact on endothelial function would not be an essential for further investment, an adverse outcome might prompt more cautious progression. Indeed, one might speculate that had a clinical endothelial function study been conducted at an earlier stage of the Torcetrapib development program, an adverse endothelial "signal" might have been detected at that point prompting a more cautious evaluation of the situation-given what we now know about the adverse impact of this agent on blood pressure and the renin-angiotensin system.

*4.4. Endothelial "Stress Testing": Opportunities to Gain Additional Insights into Vascular Pathophysiology.* Recently, several experimental clinical models of acute endothelial dysfunction have been developed which allow exploration of endothelial behaviour in response to relevant, acute pathophysiological stimuli. These include ischemia reperfusion, inflammation, and acute psychophysiological stress. Application of these models either to explore drivers of disease progression and destabilization or to develop strategies to protect the cardiovascular system against these pathophysiological influences is still at an early stage but clearly has great potential [75–80].

## 5. Conclusion

The endothelium has an important influence on the development of atherosclerosis, and the assessment of its function affords a valuable insight into disease processes in the arterial wall. This has been exploited in a wide range of clinical situations and has more recently been used to good effect in large prospective cohort studies. It is important to emphasize that endothelial dysfunction, although important, is only a component of the pathophysiological process of atherogenesis. For example, inflammatory, proliferative, and thrombotic pathways acting independently of the endothelium also have important influences on plaque development, destabilization, and clinical sequelae. Unfortunately, the endothelium's inherent physiological sensitivity and complexity of some of the assessment methodologies make it a less suitable parameter to guide routine clinical practice, but it remains a core component of the clinical vascular research technique portfolio. Indeed, results from endothelial function testing provide insightful short- to medium-term outcome measures for detailed mechanistic vascular studies and early-phase clinical trials.

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*Clinical Study*

# Augmentation Index Derived from Peripheral Arterial Tonometry Correlates with Cardiovascular Risk Factors

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*Background*. Augmentation index (AIx) is traditionally obtained from pressure waveforms via arterial applanation tonometry. We sought to evaluate the association between AIx obtained from peripheral arterial tonometry (PAT) with cardiovascular risk factors (CRF) and coronary artery disease (CAD). *Methods*. 186 patients were enrolled in the study. The presence or absence of CRFs and CAD was assessed in each subject. AIx was calculated by an automated algorithm averaging pulse wave amplitude data obtained via PAT. Central blood pressures were assessed in a subset of patients undergoing clinically indicated cardiac catheterization. *Results*. An association was observed between AIx and age, heart rate, systolic blood pressure, mean arterial pressure, pulse pressure, body weight and body mass index. AIx was significantly lower in patients with <3 CRFs compared to those with >5 CRFs ($P = .02$). CAD+ patients had significantly higher AIx compared to CAD− patients ($P = .008$). Area under the ROC curve was 0.604 ($P < .01$). In patients undergoing cardiac catheterization, after adjusting for age, height and heart rate, AIx was a significant predictor of aortic systolic and pulse pressures ($P < .05$) *Conclusion*. AIx derived from PAT correlates with cardiac risk factors and CAD. It may be a useful measure of assessing overall risk for coronary artery disease.

## 1. Introduction

A portion of the arterial pressure wave travelling towards the extremities is reflected back from peripheral impedance points. In healthy individuals, the reflected wave returns to the aorta during diastole. With age and in the presence of vascular disease, arterial compliance decreases, and the arteries become stiff, thereby reducing the transit time for the incident and reflected waves. Consequently, the reflected wave arrives at the aorta during systole of the same cardiac cycle augmenting the central blood pressures. This augmentation of central pressure can be quantified by augmentation index (AIx), defined as the percentage of the central pulse pressure attributed to the reflected pulse wave.

AIx has been shown to be associated with cardiovascular risk [1], predicts the presence or absence of coronary artery disease (CAD) [2, 3], and has been shown to be an independent predictor of cardiovascular events and all-cause mortality in select patient populations [4, 5]. Conventionally, AIx is obtained from pressure waveforms via applanation tonometry of the carotid or radial arteries. Recently, it has been demonstrated that AIx can be measured from digital pulse wave volumes by peripheral arterial tonometry (PAT) [6, 7], and that it is associated with ventricular-vascular coupling [8]. The value obtained by AIx derived by PAT correlates with that obtained by conventional radial applanation tonometry; however, they cannot be used interchangeably since the actual values do not match since they are obtained from two distinct vascular beds via two different methods. Little is known about the pathophysiological correlates of PAT-derived AIx. Therefore, we sought to evaluate the association between PAT-derived AIx and cardiovascular risk factors (CRFs) and/or CAD.

## 2. Methods

### 2.1. Patient Population

*Study 1.* 146 patients recruited from the Cardiology Clinic at Tufts Medical Center in Boston were enrolled in the study.

Table 1: Study population characteristics.

| | Total population ($n = 186$) | Number of cardiac risk factors | | | $P$ value |
|---|---|---|---|---|---|
| | | <3 ($n = 64$) | 3–5 ($n = 77$) | >5 ($n = 44$) | |
| Age, yrs | 59 ± 1 | 54 ± 1*† | 60 ± 1 | 61 ± 1 | .0001 |
| Females, *n* (%) | 57 (36) | 18 (51)*† | 28 (41) | 11 (13) | .01 |
| Menopause, *n* (%) | 35 (61) | 12 (67)* | 14 (50) | 9 (81)‡* | .01 |
| Heart rate, beats per minute | 68 ± 1 | 70 ± 1*† | 66 ± 1 | 64 ± 2 | .04 |
| Mean arterial pressure, mmHg | 103 ± 1 | 99 ± 2*† | 103 ± 1 | 106 ± 1 | .009 |
| Systolic blood pressure, mmHg | 128 ± 1 | 124 ± 2 | 129 ± 2 | 127 ± 2 | .18 |
| Diastolic blood pressure, mmHg | 76 ± 1 | 78 ± 1 | 76 ± 1 | 71 ± 1*‡ | .006 |
| Pulse pressure, mmHg | 51 ± 1 | 45 ± 1*† | 52 ± 1 | 56 ± 2 | .008 |
| Height, inches | 67.8 ± 0.5 | 65 ± 1*† | 68 ± 0.5 | 70 ± 0.3 | .01 |
| Weight, pounds | 192.9 ± 2.8 | 183 ± 6† | 191 ± 3† | 209 ± 4 | .02 |
| Body mass index, kg/m$^2$ | 29.7 ± 0.4 | 29.5 ± 1 | 29.4 ± 1.8 | 30.7 ± 0.6*‡ | .06 |
| Total cholesterol, mg/dL | 174 ± 3 | 194 ± 5*† | 168 ± 4 | 155 ± 6.3 | .03 |
| HDL cholesterol, mg/dL | 42 ± 9 | 50 ± 2*† | 39 ± 2 | 36 ± 9 | .03 |
| LDL cholesterol, mg/dL | 103 ± 3 | 116 ± 7*† | 99 ± 5 | 92 ± 5 | .04 |
| Triglycerides, mg/dL | 158 ± 10 | 131 ± 11 | 170 ± 19 | 152 ± 15 | .290 |
| Hypertension, *n* (%) | 96 (52) | 6 (17) | 31 (46)†‡ | 59 (71)*‡ | .03 |
| Diabetes mellitus, *n* (%) | 44 (24) | 0 (0) | 9 (14)‡† | 35 (42)*‡ | .0001 |
| Hypercholesterolemia, *n* (%) | 109 (58) | 2 (6) | 37 (54)‡† | 70 (84)*‡ | .0001 |
| Smoke, *n* (%) | 82 (44) | 7 (20) | 27 (40) | 48 (58) | .201 |
| Family history, *n* (%) | 65 (35) | 5 (14) | 23 (34)‡† | 37 (45)‡ | .001 |
| Coronary artery disease, *n* (%) | 102 (55) | 7 (11) | 53 (68)‡† | 42 (95)‡ | .001 |
| Augmentation index, (%) | −4.86 ± 1.4 | −5.15 ± 2.09† | −3.60 ± 1.69 | 5.65 ± 2.55 | .02 |

‡ Significantly different from cohort with <3 CRFs.
* Significantly different from cohort with 3–5 CRFs
† Significantly different from cohort with >5 CRFs.

Subjects were instructed to fast overnight and to refrain from smoking, caffeine, or alcohol on the day of testing. Vasoactive medicines were withheld for a period of 12 hours prior to testing.

*Study 2.* 40 consecutive patients undergoing clinically indicated nonemergent left heart catheterization were retrospectively identified for inclusion into this study. As part of the standard catheterization protocol, blood pressures were measured within the femoral artery, ascending aorta, and the left ventricle using a 6-French end hole fluid-filled catheter and a pressure manometer prior to injection of any contrast agent. Pressures within the ascending aorta were considered as the central blood pressure (CBP) in the study.

The presence or absence of the following cardiovascular risk factors was assessed in all the subjects from both substudies: male gender, hypertension (systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or being on an antihypertensive medication), hyperlipidemia (serum cholesterol >220 mg/dL or taking lipid-lowering medication), diabetes mellitus (fasting blood glucose >140 mg/dL or on oral hypoglycemics or insulin), family history of CAD (first-or second-degree relatives with premature CAD), postmenopausal women, and smoking (having smoked at least five times per day within last month). CAD was defined as the presence of ischemia or infarction on single-photon emission-computed tomographic (SPECT) nuclear myocardial perfusion imaging or >50% stenosis of an epicardial coronary artery by angiography.

Patients with moderate to severe heart failure (NYHA class III-IV, LVEF < 35), hemodynamic instability, peripheral arterial disease (Ankle-brachial Index <0.9), and finger deformities were excluded from the study. This study was approved by the Institutional Review Board at Tufts Medical Center. A written informed consent was obtained from all subjects.

*2.2. Augmentation Index Measured by Peripheral Arterial Tonometry (PAT-AIx).* Prior to collecting vascular measures, all study subjects rested in a quiet dark room for a period of 10 minutes. Post-acclimatization, the vascular data was recorded by PAT (EndoPAT2000, Itamar Medical Ltd, Caesarea, Israel) for a period of 5 minutes with the patient in supine position. The procedure for obtaining AIx is essentially the same as described in previous reports [9]. The probes, lined by inflatable balloons, are connected to isolated volume reservoirs within the machine, which buffer pressure changes and provide a uniform pressure field. Also, the counter-pressure imparted by the balloon prevents distal venous distension and blood pooling, which might induce a local venoarteriolar vasoconstriction reflex [10, 11]. The PAT system is designed to unload arterial wall tension and increase the range of arterial wall motion without inducing

potentially confounding vasomotor changes. Pressure sensors within this cap capture beat-by-beat finger pulse wave amplitude (PWA) which is then filtered, amplified, and graphically displayed on a computer screen. A computerized algorithm has been generated to automatically identify peak pressures and inflection point as previously described by Kelly et al. [12] and Takazawa [13]. PAT-AIx is calculated by averaging the PWA data over an epoch of 3.5 minutes and is calculated by the following formula: PAT-AIx = $(P_2 - P_1) \times 100 / P_1(\%)$, where $P_1$ = pulse pressure and $P_2$ = pressure corresponding to the inflection point.

*2.3. Statistical Analysis.* Data were entered into MS-Excel and analyzed with SPSS (SPSS Inc., Chicago, Ill, USA). Data are expressed as mean ± SEM. A priori significance was set at $P < .05$. Normality of distributions was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests. Continuous variables were analyzed according to the student $t$-test and Pearson correlation. Dichotomous variables were analyzed by means of the $\chi^2$ analysis. Cardiac risk factors (age >45 yrs in men and >55 yrs in women, male gender, menopause, hypertension, hypercholesterolemia, cigarette smoking, diabetes mellitus and family history of CAD) were tabulated as nominal variables. Depending upon the total number of CRFs per patient, the study population was divided into tertiles, including those with <3 CRFs, 3–5 CRFs, and >5 CRFs. Analysis of variance (for parametric data) with Scheffe post hoc testing was performed to assess difference in continuous variables between groups. A stepwise multiple regression analysis was performed to examine predictors of AIx. Binary logistic regression analysis (0.05 to enter, 0.10 to remove) was used to evaluate for relationship between AIx and CAD and to evaluate for potential confounders including age, gender, height, heart rate, presence of hypertension, hypercholesterolemia, diabetes mellitus, tobacco use, and family history of CAD. A receiver operator characteristic (ROC) curve was generated to determine the predictive power of AIx for CAD.

## 3. Results

*3.1. Subject Population.* 186 subjects (129 men, 57 women) with an average age of 59 ± 1 years were enrolled. 61% of women were postmenopausal, and 35% were on hormone replacement therapy. 55% patients had CAD, 52% patients had hypertension, 24% were diabetic, 58% had hypercholesterolemia, 44% were smokers, and 35% had family history of CAD. Detailed characteristics of the study population are described in Table 1.

*3.2. Relationship between Peripheral AIx and Cardiac Risk Factors.* A significant association was observed between PAT-AIx and age ($r = 0.265$, $P < .0001$), heart rate ($r = -0.299$, $P < .0001$), diastolic blood pressure ($r = -0.285$, $P < .0001$), mean arterial pressure ($r = 0.170$, $P < .045$), and pulse pressure ($r = 0.198$, $P = .012$). The relationship between PAT-AIx and age best fitted a quadratic regression model ($P = .01$). AIx was not associated with diabetes

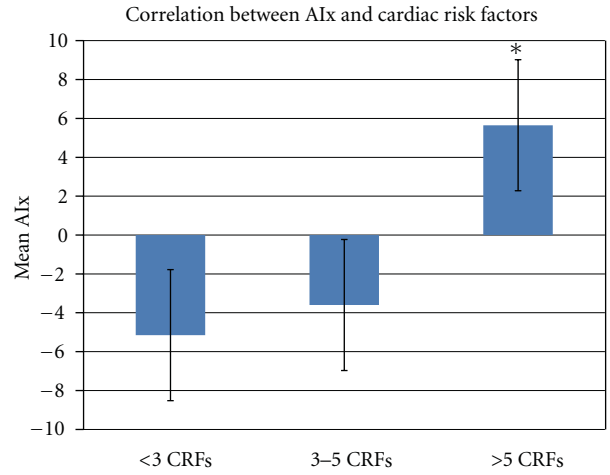


Figure 1: Patients with >5 cardiac risk factors had a significantly higher PAT-AIx compared to those with <3 CRFs ($P = .02$). PAT-AIx in patients with 3–5 cardiac risk factors was not significantly different from those with <3 or >5 CRFs.

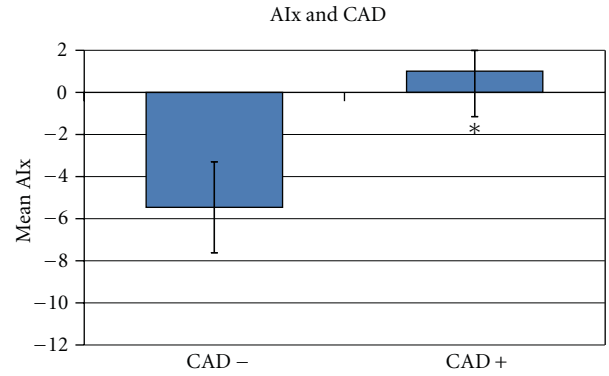


Figure 2: CAD− patients have significantly lower PAT-AIx values than CAD+ patients ($P = .008$).

mellitus, smoking status, or family history of CAD. Similarly, no significant correlation was observed between PAT-AIx and systolic blood pressure, gender, height, and lipids. Stepwise multiple regression analysis identified age, heart rate, diastolic blood pressure, weight, and cigarette smoking as predictors of PAT-AIx. Analysis of variance (Scheffe post hoc testing) revealed a significant difference in AIx between patients with <3 CRFs (−5.15 ± 2.09%) and those having >5 CRFs (5.65 ± 2.55%, $P = .02$, Figure 1). PAT-AIx in patients with 3–5 cardiac risk factors was not significantly different than in those with either <3 or >5 CRFs.

*3.3. Relationship between Peripheral AIx and CAD.* PAT-AIx was significantly higher amongst CAD+ patients (1.01 ± 1.6%) as compared to CAD− patients (−5.46 ± 1.7%, $P = .008$, Figure 2). To examine the ability of PAT-AIx to predict CAD+, the area under the ROC curve yielded a value of 0.604 ($P < .01$, Figure 3). After dividing the study population into tertiles based on the PAT-AIx values, there was a stepwise increase in prevalence of CAD with an increase in PAT-AIx ($P < .05$, Figure 4). However, after adjusting

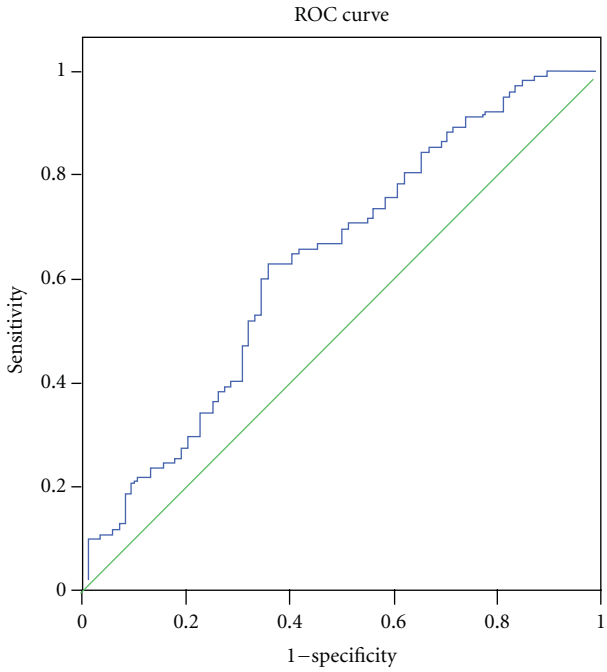


Figure 3: The classification performance of peripheral AIx, assessed by generating a receiver operated characteristic curve, revealed an AUC of 0.604 ($P < .01$).

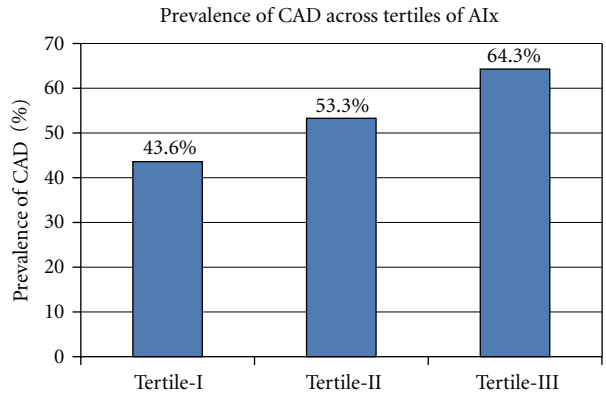


Figure 4: Prevalence of CAD in study patients divided into tertiles based on their PAT-AIx results. The highest number of CAD cases was found in the tertile with the highest PAT-AIx values (Tertile III). Tertile I, having the lowest PAT-AIx values, had the lowest number of CAD cases.

for potential confounders, including age, sex, heart rate, smoking, diabetes, hypertension, and hypercholesterolemia, PAT-AIx was not significantly different between these two groups.

*3.4. Relationship between Digital AIx and Central Blood Pressures.* A positive correlation was noted between peripherally derived AIx and centrally measured blood pressures. PAT-AIx correlated with aortic systolic pressure ($r = 0.480$, $P = .002$, Figure 5), and aortic pulse pressures ($r = 0.455$, $P = .004$, Figure 6). After adjusting for age, height, and heart rate, AIx remained a significant predictor of aortic systolic

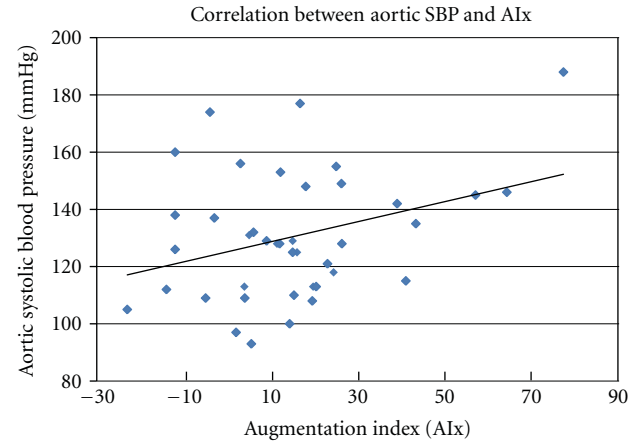


Figure 5: Positive correlation between PAT-AIx and aortic systolic blood pressure ($r = 0.480$, $P = .002$).

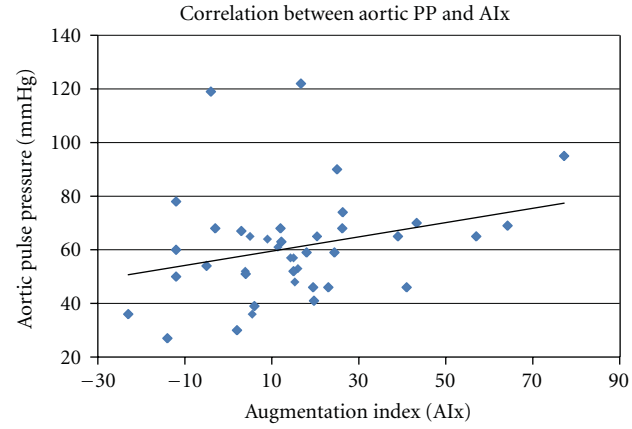


Figure 6: Positive correlation between PAT-AIx and aortic pulse pressure ($r = 0.455$, $P = .004$).

pressure ($\beta = 0.449$, 95% CI 0.187–0.712, $P = .001$) and aortic pulse pressure ($\beta = 0.424$, 95% CI 0.042–0.805, $P = .0031$).

*3.5. Relationship between Peripheral AIx and Medications.* Analysis focusing on effects of medications on peripheral augmentation index revealed a significant association between PAT-AIx with beta-blockers ($P = .018$) and ACE inhibitors ($P = .03$). There was no association between PAT-AIx and calcium blockers, angiotensinogen receptor blockers, nitrates, statins, digoxin, diuretics, oral hypoglycemics, insulin, thyroid hormone supplements, antidepressants, and steroids.

## 4. Discussion

Our study was undertaken to evaluate the association of peripheral AIx and cardiovascular risk factors. Similar to what has been found with AIx derived from applanation tonometry [1], PAT-derived AIx correlates with age, weight, BMI, heart rate, diastolic blood pressure, pulse pressure,

and mean arterial pressure. PAT-AIx was also significantly related to age. Previous studies have noted that the increase in AIx with age is not linear [1]. In alignment with previously published data, a quadratic model best illustrates the relationship between age and PAT-AIx. AIx values increased with age up to 65–70 years of age. Beyond that, the AIx values tended to plateau and then decrease shortly after that. It has been observed that AIx measured by conventional applanation tonometry begins to plateau at the age of 60 years. This observed difference can be explained in part by impedance matching. Impedance matching may differ in the hand circulation versus the aorta as there may not be changes in digital endothelial function with aging [14].

PAT-AIx was associated with the cumulative CRFs burden in this study population. Patients with >5 CRFs had a significantly higher PAT-AIx as compared with those having only a few CRFs. Similarly, when the prevalence of CAD was assessed across tertiles of PAT-AIx, it was noted that patients in the highest tertile had a significantly higher prevalence of CAD as compared to those in the lower tertiles.

PAT-AIx was able to discern the presence or absence of CAD amongst a heterogeneous patient population with varying in cardiovascular risk. The classification performance of peripheral AIx was assessed by generating a receiver-operated characteristic curve which revealed an AUC of 0.604. However, after adjusting for traditional risk factors such as age, sex, heart rate, height, weight, hypertension, diabetes mellitus, hypercholesterolemia, smoking, and family history of premature coronary heart disease, AIx lost its statistical significance. Thus, PAT-derived AIx is not an independent predictor of CAD in this population.

The importance of assessing central blood pressure lies in the fact that traditionally measured brachial artery blood pressure is often a poor representation of actual central pressures. Due to pressure wave amplification, the brachial systolic pressure can be up to 20 mmHg higher than the aortic systolic pressure [15–17]. Since the left ventricle works against pressures within the ascending aorta, aortic pressures play a significant role in the pathophysiology of cardiac disease. As such, aortic systolic and pulse pressures can be considered as novel cardiovascular risk factors since they are predictors of cardiovascular events, target organ damage, and mortality [18]. Compared to brachial systolic and pulse pressure, aortic pulse pressure appears to be a better predictor of the presence and extent of coronary atherosclerosis [19, 20] and restenosis after coronary intervention [21]. Furthermore, a reduction in central blood pressure results in a reduction in cardiac hypertrophy, vascular thickness, and adverse cardiac events [22–24]. For example, a 10 mmHg reduction in aortic pulse pressure has been shown to translate into a 13–15% reduction in CV events and all-cause mortality [25, 26]. In alignment with data published by Munir et al. [27], our study reveals that AIx measured by PAT also closely correlates with central aortic blood pressures.

We do acknowledge certain limitations in this study. While it has been suggested that arterial stiffness and augmented pressure from wave reflections make the most significant contributions to the digital volume pulse inflection point, vascular correlates or PAT-AIx remain unexplored. CAD was defined on the basis of diagnosis with angiography and/or positive SPECT imaging. It is true that SPECT imaging results do not always correlate with severity of coronary artery stenosis. However, we chose stress testing because it provides physiologic information regarding ischemia and prior infarction. While SPECT imaging does not confer 100% sensitivity and specificity, this technique is a reliable, noninvasive assessment of myocardial ischemia/infarction. In Substudy 2, central pressures were obtained using conventional fluid-filled catheters, rather than high-fidelity pressure transducers. In addition, AIx was not obtained simultaneously during catheterization when actual central pressure values were obtained. Rather, PAT was performed at the bedside following cardiac catheterization.

## 5. Conclusion

PAT has been under investigation over recent years as a technique for assessment of vascular endothelial function. The present data suggest that this simple, noninvasive technique may also provide information regarding augmentation index. Our findings suggest that finger-derived AIx may be comparable to applanation tonometry for measurement of augmentation index and evaluation of cardiovascular risk. However, as observed by Haller et al. [6], although the values of AIx obtained by PAT correlate with that obtained by conventional radial applanation tonometry, they cannot be used interchangeably since the actual numbers do not match. Additional research is needed to identify the underlying vascular physiology modulating PAT-AIx. Further validation is warranted in large scale studies comparing PAT-AIx with that obtained by the current gold standard radial applanation tonometry in a wider patient population.

## Disclosures

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*Review Article*

# Vascular Stiffness and Increased Pulse Pressure in the Aging Cardiovascular System

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Aging leads to a multitude of changes in the cardiovascular system, including systolic hypertension, increased central vascular stiffness, and increased pulse pressure. In this paper we will review the effects of age-associated increased vascular stiffness on systolic blood pressure, pulse pressure, augmentation index, and cardiac workload. Additionally we will describe pulse wave velocity as a method to measure vascular stiffness and review the impact of increased vascular stiffness as an index of vascular health and as a predictor of adverse cardiovascular outcomes. Furthermore, we will discuss the underlying mechanisms and how these may be modified in order to change the outcomes. A thorough understanding of these concepts is of paramount importance and has therapeutic implications for the increasingly elderly population.

## 1. Hallmarks of the Aging Cardiovascular System

Aging leads to a multitude of changes in the cardiovascular system including increased vascular stiffness. In fact, age-related increases in blood pressure are mainly attributable to an increase in systolic blood pressure while maintaining or having a slight decrease in a diastolic blood pressure. This leads to a widening in pulse pressure (difference between systolic and diastolic blood pressure) [1]. Systolic hypertension is so closely related to aging that people 65 years of age have a 90% chance of developing hypertension in their lifetime [1]. Isolated systolic hypertension is the most common subtype of hypertension in the middle-aged and elderly and is tightly coupled to increased arterial stiffness and pressure augmentation by reflected waves. Other causes of a widened pulse pressure, including severe anemia, aortic insufficiency, thyrotoxicosis or arteriovenous shunting, are much more uncommon.

The arterial system has two major functions. Firstly, it acts as a conduit to deliver oxygenated blood and nutrients to the organs. Secondly, it provides a cushion to soften the pulsations generated by the heart such that capillary blood flow is almost continuous. The human body is highly adapted to achieve those functions. The composition of the arteries, especially the media, changes significantly as one moves from proximal (central large arteries, e.g., aorta and its major branches) to distal (peripheral, predominantly muscular arteries, e.g., brachial or radial). While the predominant fibrous elements in the thoracic aorta contain mainly elastin, the more distal arteries contain mainly collagen. This difference is vital for the central vessels to maintain their Windkessel function of cushioning pulsatile blood flow. With aging disruption of the cross-linking of elastin molecules leads to weakening of the elastin array with predisposition to mineralization by calcium and phosphorous, all of which lead to increased arterial stiffness [2, 3]. The widening pulse pressure seen with aging is a direct surrogate of arterial stiffness. The increase in vascular stiffness has direct implications for ventricular-arterial coupling (interaction of the heart with the systemic vasculature) [4]. The increase in systolic blood pressure increases the systolic workload of the left ventricle and increases left ventricular end-systolic stiffness and reduces diastolic compliance [4]. This leads to increased oxygen consumption, left ventricular hypertrophy, and potentially subendocardial ischemia due to imbalance in myocardial oxygen supply and demand.

## 2. Vascular Stiffness: Mechanisms

The normal young vascular tree, particularly the aorta, has the ability to cushion the pulsatile ventricular ejection and to transform it into almost continuous flow [5]. This phenomenon is often described as the Windkessel function and requires a high degree of aortic compliance [6], defined as a change in volume in response to a change in pressure ($C = \Delta V/\Delta P$). Vascular stiffness or elasticity is the reciprocal of compliance. This needs to be distinguished from (i) resistance which characterizes the relationship between mean pressure and flow and (ii) impedance which is a measure of how much a structure resists motion when subjected to a given force. In oscillating systems, instantaneous measurements are also influenced by those that immediately precede them.

The elasticity of a given arterial segment is not constant but rather depends on its distending pressure [7]. A higher distending pressure leads to an increase in recruitment of collagen fibers and therefore a reduction in elasticity [8]. This distending pressure is determined by the mean arterial pressure and must be considered whenever measurements of arterial stiffness are made. In addition to elastin, arterial wall smooth muscle bulk and tone influence arterial stiffness. Thus, the endothelium because of its capacity to modulate smooth muscle tone modulates stiffness. Moreover, vessel diameter also influences the stiffness of vessels. In general smaller vessels are relatively stiffer than bigger vessels because of their smaller radius [9]. A large vessel can accept a larger volume for the same change in distending pressure and thus has a greater compliance. Furthermore, wall composition varies with size, with the media of large central vessels' composed mainly from elastin, while peripheral conduit arteries contain relatively more collagen. With aging, this structure of the arterial wall changes as a consequence of fractures of the elastic lamina, loss of muscle attachments, increase in collagen fibers, local inflammation, infiltration of vascular smooth muscle cells and macrophages, fibrosis, deposition of mucoid material, focal media smooth muscle cell necrosis, and calcification. The intima-medial thickness triples between the ages 20 and 90 [10, 11]. A major component of this compositional change with aging is a consequence of elastin fracture, with elastin being progressively replaced by collagen [12]. This results in major age-related changes in the vasculature: it increases arterial stiffness leading to increases in systolic blood pressure and a widening pulse pressure. Furthermore these changes result in arterial dilatation as weight bearing elastin breaks down [2, 3].

Stiffness is also increased by the accrual of advanced glycation end (AGE) products [14]. These result from irreversible nonenzymatic glycation of proteins (e.g., collagen) [15]. Cross-linking and AGEs formation can also involve elastin, degrading the elastic matrix of the vessel wall [16]. Furthermore AGE increases the formation of oxygen radicals, proinflammatory cytokines, growth factors, and vascular adhesion molecules [17]. Those mediators increase vascular stiffness via matrix metalloproteinase, increasing smooth muscle tone, attenuating vasodilation, and promoting atherosclerotic plaques [18–21]. In a recent clinical trial by Kass et al., the nonenzymatic breaker of advanced glycation end-product crosslinks ALT-711, has been shown to improve total arterial compliance in aged humans with vascular stiffness, and may therefore provide a novel therapeutic approach for this abnormality [22].

In addition to the aforementioned changes, vascular smooth muscle tone and endothelial signaling exert a significant effect on vascular stiffness [17]. Mechano-stimulation can directly alter vascular tone by cell stretch, changes in calcium signaling, oxidative stress, and nitric oxide production [24–26]. The major mediator of endothelium-dependent vasorelaxation is nitric oxide (NO) [27]. It is derived from L-arginine by NOS (nitric oxide synthetase) [28]. NOS uncoupling, the generation of reactive oxygen species instead of NO [29], contributes to age-related endothelial dysfunction [30], increased vascular stiffness, slower ventricular relaxation [31], and atherosclerosis [32], all of which increase PWV. NOS uncoupling can have several etiologies including limited substrate (arginine) or cofactor (Tetrahydrobiopterin) availability, as well as a recently identified posttranslational modification by the enzyme glutathionylation (oxidized glutathione) [33–35]. In addition to its vasoactive effects, NO modulates the activity of the matrix crosslinking enzyme transglutaminase (TG) via S-nitrosylation, also leading to increases in arterial stiffening [36, 37]. Other mechanisms recognized as contributing to the development of increased vascular stiffness in aging include a decrease in NOS expression [38], an increase in xanthine oxidase activity [39, 40], and an increase in reactive oxygen species [39, 41], while stiffening itself can lead to a decrease in NOS activity [42].

## 3. Measurements of Vascular Stiffness

The arterial pressure waveform is a composite of two waveforms, namely, a forward pressure wave due to ventricular contraction and ejection of blood into the aorta and a backward wave created by reflections at vascular branching points and at points of impedance mismatch (branch points, abrupt change in vessel diameter, and high resistance arterioles; Figure 1) [23]. The speed of travel of this wave along the artery is called pulse wave velocity (PWV) [13]. In young vascular beds, the reflected wave arrives back at the aortic root during diastole [12]. Increased arterial stiffness, as that occuring for example with aging, results in an increase in PWV and the reflected wave arrives back to the central circulation during systolic ejection. This adds to the forward wave, augmenting systolic blood pressure and widening pulse pressure. This amplification can be quantified by measuring the augmentation index utilizing applanation tonometry. The augmented component is represented by the difference between the first and second systolic peaks, and the augmentation index is defined as the ratio of this component to pulse pressure (Figure 2). Therefore the augmentation index represents a complex measure of wave reflection and incorporates arterial stiffness but is not in itself a measure of stiffness [43]. Another index of vascular stiffness

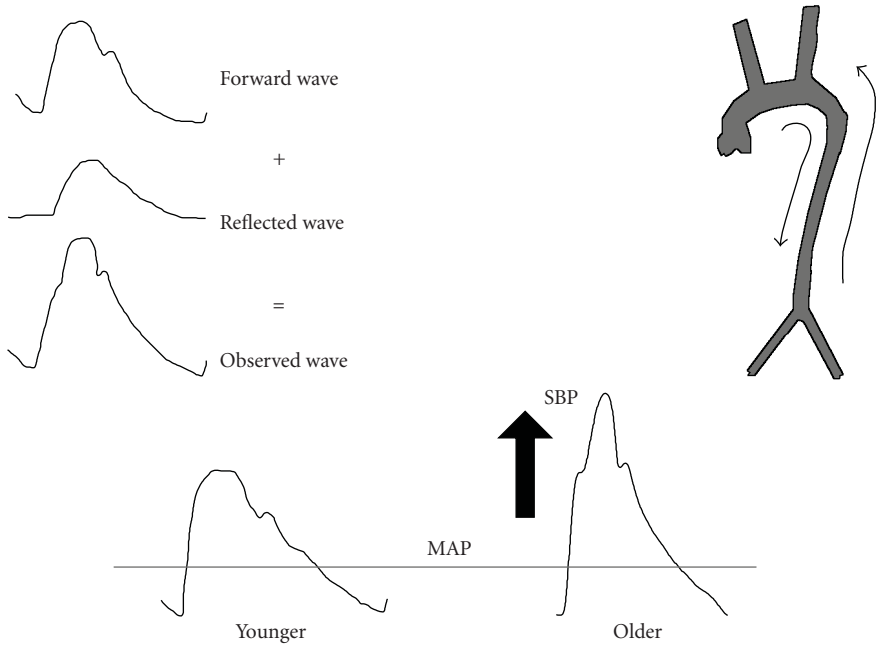


Figure 1: This figures illustrates the composition of the arterial pressure waveform from a combination of a forward pressure wave created by ventricular contraction and a reflected wave. MAP: mean arterial pressure; SBP: systolic blood pressure (see [13]).

is pulse pressure amplification, which can be quantified as the ratio of the amplitude of proximal pulse pressure and distal pulse pressure [44]. Of note, there is recent evidence by Mitchell et al. that questions the dominant role of the reflected pressure wave. They studied an unselected community-based population and suggested that the late-life increases in pulse pressure are attributable predominantly to an increase in forward pressure wave amplitude and that wave reflection plays only a minimal role [45]. Irrespective of which factor contributes the most, aging is associated with systolic hypertension, increased pulse pressure, and increased ventricular loading conditions. Augmentation index, pulse pressure amplification, and especially PWV are increasingly utilized as a marker of cardiovascular disease [46]. PWV increases with stiffness and is defined by the Moens-Korteweg equation: PWV = $\sqrt{(Eh/2pR)}$, where $E$ is Young's modulus of the arterial wall, $h$ is wall thickness, $R$ is arterial radius, and $p$ is blood density. Despite this rather complex formula, measurement of PWV is relatively simple. The arterial pulse wave is recorded at both proximal artery site (e.g., common carotid) and distal artery site (e.g., femoral or brachial) [47]. The time delay between the arrival of the pulse wave is obtained either by simultaneous measurement or by gating to the peak of the R-wave of the ECG. The distance is measured over the body surface and the PWV is calculated as distance/time (m/s). The measurement of distance between the two points is only an estimate of the true distance given the individual body habitus. Arterial pulse wave can be detected by pressure sensitive transducers, Doppler ultrasound, or applanation tonometry. Another noninvasive but more complex option is to measure PWV by MRI. This has the advantage of determining the exact path of the pressure wave but is time consuming, impractical clinically, and very costly.

Central vascular stiffness can be assessed noninvasively by measuring pulse wave velocity, assessing pressure waveforms, or measuring pulse pressure. Caution must be exercised when utilizing pulse pressure as an index of central stiffness. In peripheral arteries, reflection sites are closer, and this results in a greater amplification of the pressure wave in peripheral arteries. Hence in young individuals with healthy vessel properties, peripheral pulse pressure is normally greater. In elderly individuals, including patients with hypertension or diabetes, central pulse pressure increases because of altered stiffness properties, and central pulse pressure can approach and indeed equal peripheral pulse pressure.

## 4. Vascular Stiffness Measurements as a Prognostic Indicator

Many large epidemiologic studies, including one of the Framingham cohorts including 2,232 patients, established the role of systolic blood pressure and pulse wave velocity as a predictor for adverse cardiovascular events [48, 49]. Additional studies have demonstrated that brachial pulse pressure is a strong and independent predictor for congestive heart failure and stroke in hypertensive patients and in the general population [50–54]. In the ABC study with 2,488 elderly participants, higher PWV was associated with higher cardiovascular mortality, congestive heart failure, and stroke after adjustment for age, gender, race, systolic blood pressure, known cardiovascular disease, and other recognized cardiovascular risk factors [55]. Similar results were obtained in the

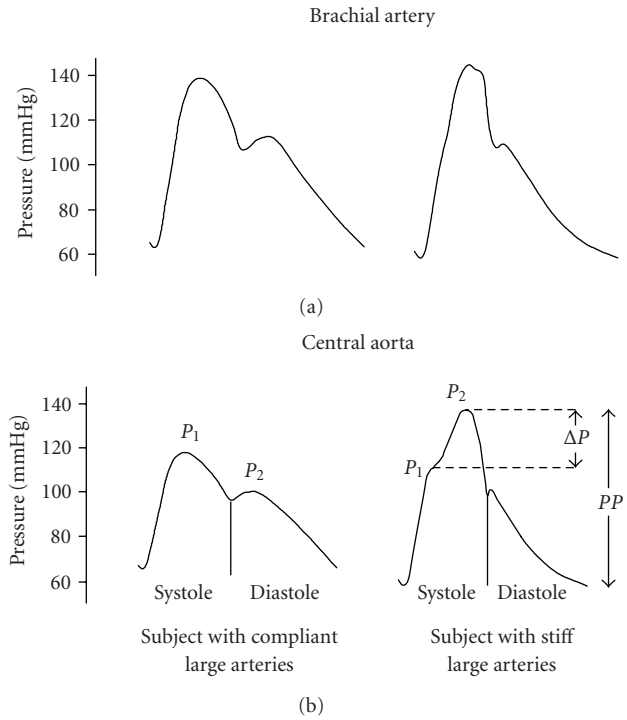


Figure 2: Schematic representation of pulse pressure amplification. Pressure tracings from the brachial artery and central aorta are shown, from a young individual with a highly compliant vasculature tree (left) and from an old one with stiff vessels (right). Despite similar brachial blood pressures, central blood pressures vary considerably. In young individuals, $P_1$ (which coincides with systolic blood pressures) marks the outward traveling blood pressure wave, while $P_2$ represents the arrival of the reflected pressure wave in diastole, augmenting diastolic blood pressure, and coronary filling. Pulse pressure augmentation in old, stiff vessels leads to a significant increase in $P_2$ as compared to $P_1$, as wave reflection occurs earlier and faster, leading to an augmentation in systolic blood pressure. Augmentation index is calculated as the difference between the second ($P_2$) and first ($P_1$) systolic peaks (delta $P$) as a percentage of pulse pressure (see [23]).

Rotterdam study that included 2,835 healthy subjects, which showed that aortic pulse wave velocity is an independent predictor of coronary artery disease and stroke [56]. The Monica study with 1,678 participants aged 40–70 and a 10-year followup demonstrated that increased aortic pulse wave velocity predicted a composite of cardiovascular outcomes [57]. This was recently replicated by Wang et al. studying 1,980 outpatients (mean age 50 ± 13 years) with a 10-year followup, showing that PWV was significantly associated with all-cause and cardiovascular mortality, independent of previous cardiovascular disease [48]. Similar results were obtained in the Strong Heart Study of 3,520 individuals, which showed that noninvasively determined central pulse pressure is a better predictor of vascular hypertrophy, atherosclerosis, and cardiovascular events than brachial blood pressure [58]. The ASCOT study of 259 participants corroborated these findings [59], as well as a study by Safar et al. on 180 patients which demonstrated that pulse pressure amplification is an independent predictor of all-cause mortality [60]. Finally, and of critical importance, the Dublin Outcome Study of 11,291 individuals which included middle-aged participants without hypertension clearly demonstrated that an arterial stiffness index and pulse pressure were independent predictors of cardiac mortality and stroke [61]. This was again reinforced by an even larger study in Paris, involving 125,151 middle-aged patients over 12 years without cardiovascular disease undergoing a regular health checkup [62]. The authors showed that brachial pulse pressure, calculated carotid pulse pressure, and carotid-brachial pulse pressure amplification all predict cardiovascular mortality, with carotid-brachial pulse pressure amplification being the strongest predictor [62].

In addition to being a predictor of cardiovascular events in the general population, pulse pressure has also been shown to be independently and significantly associated with renal dysfunction and renal failure after coronary artery bypass graft surgery [63]. In this international prospective multicenter clinical trial involving 4801 patients, every 20 mmHg increase in perioperative pulse pressure above 40 mmHg was associated with significant increase in the rate of renal dysfunction or renal failure [63]. Furthermore, elevated pulse pressure has been shown to be a predictor of stroke after cardiac surgery [64]. More recently pulse pressure has also been shown to be an independent predictor of cardiovascular deaths in a similar cohort after coronary artery bypass graft surgery [65].

A recent systematic review and meta-analysis by Vlachopoulos et al. evaluated 17 longitudinal studies that studied the effects of aortic PWV on a total of 15,877 patients for an average of almost 8 years [46]. They showed that the pooled relative risks increase in a stepwise fashion from the first to the third tertile. Furthermore they divided their analyses into three categories: (a) high versus low aortic PWV, (b) increase in aortic PWV by 1 m/s, and (c) increase in aortic PWV by 1 standard deviation, all of which show that in high-risk (subjects with coronary artery disease, renal disease, hypertension or diabetes) and low-risk subjects (general population) there is an increase in total cardiovascular events, cardiovascular mortality, and all-cause mortality [46] (Figure 3). They therefore conclude that PWV is a very strong predictor of cardiovascular events and all-cause mortality and supported the implementation of aortic pulse wave velocity into clinical practice [46]. This study was followed by an initiative to determine and establish reference values for pulse wave velocity in healthy subjects that can now be used to identify people at higher risk in a certain age group [66].

## 5. Vascular Stiffness and Treatment/Management Strategies

Central pulse pressure is a strong predictor of adverse cardiovascular outcomes. The CAFE study which involved 2,199 patients in five centers evaluated two different blood pressure regiments [67]. A combination of amlodipine and an ACE inhibitor was superior in lowering central pressures compared to a combination of atenolol and a thiazide diuretic

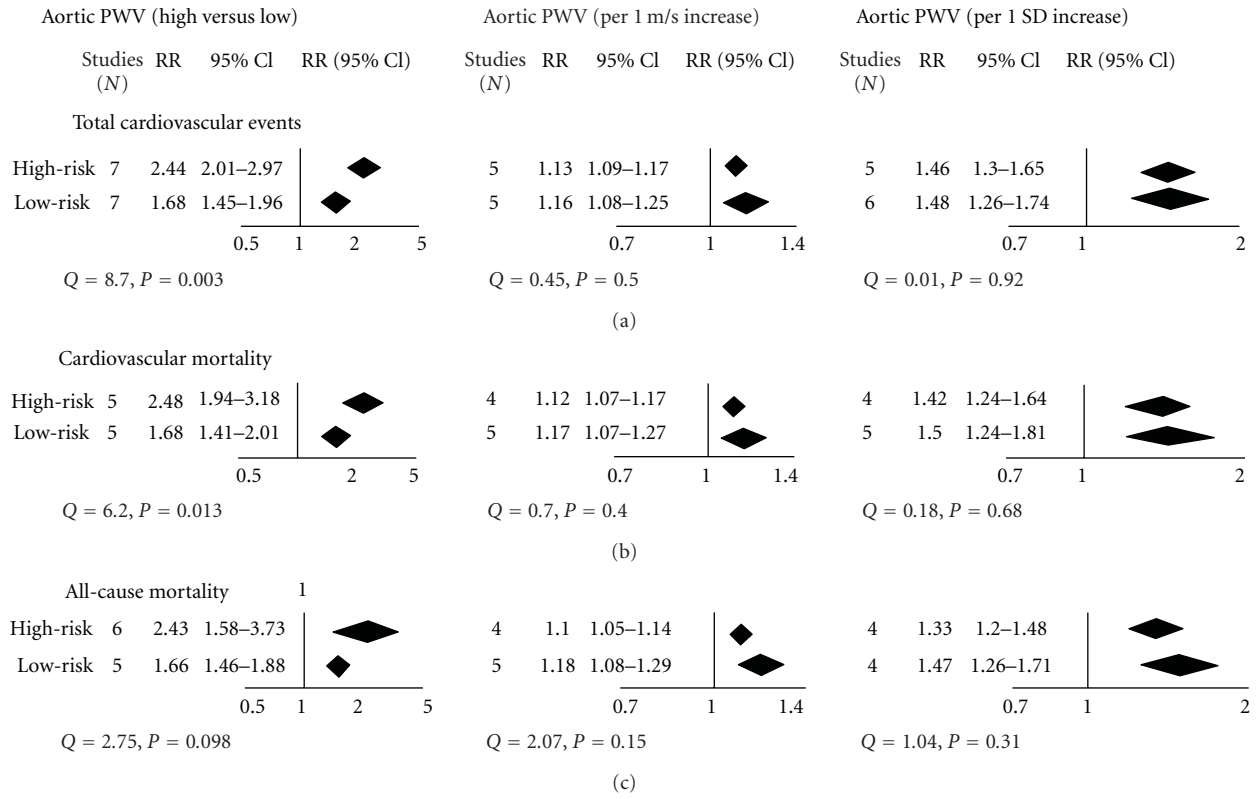


Figure 3: This figure shows pooled RR and 95% CI for aortic PWV and total CV events (a), CV mortality (b), and total mortality (c), according to baseline risk and disease state. Data are provided for high versus low aortic pulse wave velocity (PWV) (left column), increase in aortic PWV by 1 m/s (middle column), and increase in aortic PWV by 1 SD (right column) (see [46]).

[67]. This was true despite the fact that both medical regiments lowered brachial blood pressures to the same extent. Moreover, long-term cardiovascular outcomes were superior in the group treated with a combination of amlodipine plus an ACE inhibitor. The authors suggested that differences in central aortic pressures may be a potential mechanism underlying the different clinical outcomes between different blood pressure treatments. Subsequent analyses showed that the main reason underlying the apparent lack of effect of beta-blockers plus a diuretic on central pressures was that beta-blockers lowered heart rate to a greater degree than amlodipine plus ACE inhibitors. This leads to a higher augmentation index and therefore higher central aortic pressures. The reasons for this are twofold: (i) a reduction in heart rate leads to an increase in stroke volume to maintain cardiac output, which when ejected into a stiff aorta causes an increase in systolic blood pressure, (ii) a lower heart rate prolongs the cardiac cycle duration, which delays the time to peak of the outgoing pulse wave and causes the reflected wave to return in late systole, resulting also in an increase in systolic blood pressure. After these investigators adjusted for heart rate, the differences in central systolic and pulse pressures between treatment arms were no longer significant and the differences in indices of central blood pressure augmentation were minimal.

Research in the area of vascular stiffness is motivated by the desire to understand and interrogate underlying mechanisms and thus to modulate stiffness and the resultant cardiovascular sequelae. Many interventions involving lifestyle and dietary modifications, for example, smoking cessation [68], use of unsaturated fatty acids [69], isoflavones (abundant in soy beans) [70], reduced dietary salt intake [71], regular cardiovascular exercise [72], and moderate alcohol consumption [73, 74] have all been linked to reducing vascular stiffness. Other strategies involve pharmacologic interventions like calcium channel blocker, diuretics, ACE inhibitors, beta-blockers, nitrates, phosphodiesterase-5 inhibitors, and statins. Even though all these therapies lower blood pressure, their effect on arterial stiffness is only modest. A substudy of the REASON trial by de Luca et al. on 146 subjects showed that a combination of ACE inhibitor and a nonthiazide sulphonamide lowered left ventricular mass, as well as central and brachial blood pressure to a greater extend than atenolol therapy [75]. Similar results were seen in a study by Morgan et al. on 32 elderly patients; they showed that augmentation pressures on beta-blockers was greater than with placebo, while augmentation pressures following ACE inhibitors, calcium channel blockers, or diuretics were significantly less compared to placebo [76]. The lowest central aortic pressures were achieved with calcium-blocking drugs and diuretics [76]. In a small study by Hayashi et al., on 24 normotensive elderly subjects, administration of an ACE-inhibitor ameliorated age-related increases in carotid arterial

stiffness [77]. Nitrates, on the other hand, did not influence aortic stiffness even though they reduced pulse pressure by selective venodilatation and attenuation of peripheral wave reflection [78]. Phosphodiesterase-5 inhibitors like sildenafil work similarly to nitrates and also reduce wave reflection and lower pulse pressure, but without the side effects of nitrates [79]. Arterial stiffness could potentially be improved with HMG-co A enzyme inhibition by statins, although this effect is at least partially attributable to a reduction in LDL cholesterol and NOS activation [80, 81]. However, a recent meta-analysis, which included 471 participants, was unable to demonstrate that statins cause a decrease in arterial stiffness. The authors recommended that prospective randomized clinical trials should be conducted in order to reach more robust conclusions [82].

## 6. Conclusions

Aging leads to a multitude of changes in the cardio-vascular system, and it is a powerful predictor of adverse cardiovascular events. A hallmark of this process is increased central vascular stiffness, which results in an earlier return of the reflected pulse wave, adding to the forward wave and consequently augmenting central systolic blood pressure, widening pulse pressure, increasing cardiac loading conditions, and compromising vital organ perfusion. Although systolic blood pressure and pulse pressure are surrogates for this process, vascular stiffness can be measured more precisely utilizing pulse wave velocity (carotid-femoral) [43].

Vascular stiffness, an index of vascular health, has been shown to confer additional independent predictive value for adverse cardiovascular outcomes. Vascular stiffness is potentially modifiable if we understand the specific underlying mechanisms. Importantly, even in the absence of targeted therapies, an understanding of these concepts has prognostic implications, a concept already established in cardiology and emerging in the area of perioperative medicine.

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*Clinical Study*

# MicroRNA Expression Profile in CAD Patients and the Impact of ACEI/ARB

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Coronary artery disease (CAD) is the largest killer of males and females in the United States. There is a need to develop innovative diagnostic markers for this disease. MicroRNAs (miRNAs) are a class of noncoding RNAs that posttranscriptionally regulate the expression of genes involved in important cellular processes, and we hypothesized that the miRNA expression profile would be altered in whole blood samples of patients with CAD. We performed a microarray analysis on RNA from the blood of 5 male subjects with CAD and 5 healthy subjects (mean age 53 years). Subsequently, we performed qRT-PCR analysis of miRNA expression in whole blood of another 10 patients with CAD and 15 healthy subjects. We identified 11 miRNAs that were significantly downregulated in CAD subjects ($P < .05$). Furthermore, we found an association between ACEI/ARB use and downregulation of several miRNAs that was independent of the presence of significant CAD. In conclusion, we have identified a distinct miRNA signature in whole blood that discriminates CAD patients from healthy subjects. Importantly, medication use may significantly alter miRNA expression. These findings may have significant implications for identifying and managing individuals that either have CAD or are at risk of developing the disease.

## 1. Introduction

Coronary artery disease (CAD) is a major public health problem worldwide and the single largest cause of mortality in the United States, responsible for one of every six deaths (AHA Heart Disease and Stroke Statistics, 2010). CAD is caused by atherosclerosis, which is an inflammatory disease that involves multiple cell types, including circulating cells and cells in the vessel wall [1]. Despite advances in risk factor management on an epidemiological level, many individuals continue to succumb to CAD. Various blood markers associated with increased risk for death and cardiovascular endpoints have been identified, but currently very few have been shown to have a diagnostic impact or important clinical implications that would affect patient management [2]. Therefore, there is a great need for innovative biomarkers that can assess risk for CAD, assess activity of the atherosclerotic process, and guide evaluation of therapy.

Several recent studies have suggested that circulating microRNAs could be useful as biomarkers for various human disease states, including cancer [3], acute myocardial infarction [4–7], heart failure, and chronic vascular disease [8, 9]. MicroRNAs (miRNAs) are a recently recognized class of short (19–25 nt), single-stranded, noncoding RNAs that regulate an array of cellular functions through the degradation and translational repression of mRNAs that contain complementary sequences. More than 1000 human miRNAs have been identified, and, in tissues, miRNAs regulate the expression of genes involved in critical cellular processes, including differentiation, growth, proliferation, and apoptosis [10]. Importantly, miRNAs are now regarded as rheostats that fine-tune expression of proteins involved in just about every process in human cells [11].

miRNAs have been found in tissues, whole blood, serum, plasma, and other body fluids in a stable form that is protected from endogenous RNase activity [3, 12]. Although the

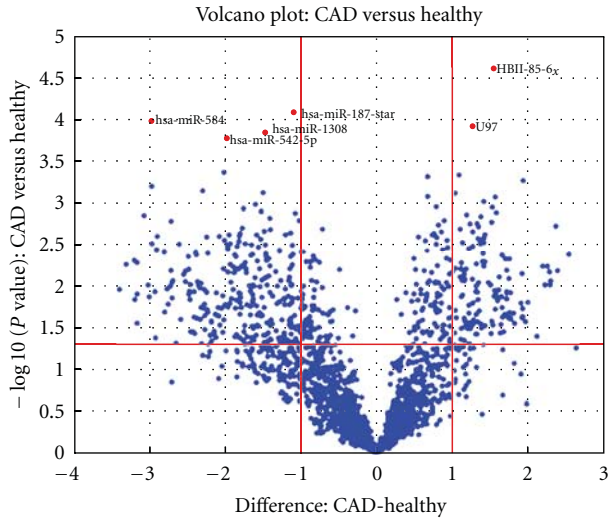


Figure 1: Volcano plot of all pairwise comparisons. Comparisons of all small RNAs assessed in microarray analysis of RNA isolated from blood of patients with CAD ($n = 5$) or healthy volunteers ($n = 5$). The volcano plot displays the relationship between fold-change and significance between the two groups, using a scatter plot view. The $y$-axis is the negative log10 of $P$ values (a higher value indicates greater significance) and the $x$-axis is the difference in expression between two experimental groups as measured in *glog2 space*. *Probes identified as significant are labeled on the plot* (*FDR t-test* <0.05). Subsequent to this analysis, miR-1308 has been removed from the Sanger database because it is a fragment of a tRNA.

biological function of miRNA is yet to be fully understood, tissue levels of specific miRNAs have been shown to correlate with pathological development of different diseases [13]. MiRNAs function as managers in gene regulatory networks, and they are distinct from other biomarkers because they have a pathogenic role in the disease process and are not merely byproducts of the disease state. This feature of miRNAs also enhances their attractiveness as therapeutic targets. Thus, miRNA expression signatures in tissues and blood have a potential role in the diagnosis, prognosis, and assessment of therapy.

In this study, we sought to compare miRNA expression in whole blood of patients with angiographically significant CAD to that of healthy aged-matched controls. We performed an initial exploratory microarray analysis in 5 cases and controls and then further examined the most highly expressed miRNAs in an additional 15 cases and controls.

## 2. Materials and Methods

### 2.1. Study Population

*2.1.1. CAD Subjects.* Study participants were recruited as part of the Emory Cardiology Biobank, consisting of 3492 consecutive patients enrolled prior to undergoing elective or emergent cardiac catheterization across three Emory Healthcare sites, between 2003 and 2008. Patients aged 20–90 years were interviewed to collect demographic characteristics, medical history, and lifestyle habits. Risk factor prevalence was determined by physician diagnosis and/or treatment for hypertension, hyperlipidemia, and diabetes.

Coronary angiograms were evaluated independently by two operators, who made visual estimation of luminal narrowing in multiple segments based on a modified form of the AHA/ACC classification of the coronary tree. Using these data, significant CAD was defined as at least one major epicardial vessel with >50% stenosis, assessed by quantitative coronary angiography. Additionally, we collected whole blood samples from patients with ≥2 risk factors for CAD, but did not have angiographically significant CAD. The study was approved by the Institutional Review Board at Emory University, Atlanta, GA, USA. All subjects provided written informed consent at the time of enrollment.

*2.1.2. Healthy Controls.* The healthy subject cohort was a random sample of employees from Emory University who were fully employed, productive people. These subjects were aged 18 and older, not taking medications, and had not been hospitalized for at least one year prior to participation. All subjects provided written informed consent at the time of enrollment.

*2.2. RNA Isolation.* Venous blood samples were drawn via antecubial venipuncture into PAXgene Blood RNA Tubes and stored at −20°C within 24 hours before RNA extraction. miRNA was isolated using the PreAnalytiX PAXgene miRNA isolation kit (Qiagen) according to the manufacturer's protocol.

*2.3. Microarray Analysis.* miRNA microarray analysis was performed by Asuragen, Inc., which uses the Affymetrix manufactured GeneChip miRNA arrays. Quality assessment of the samples was done using TaqMan assays. The microRNA targets were biotinylated using an Asuragen developed direct labeling procedure. Once labeled, the miRNA targets were hybridized overnight onto the microarrays following which the arrays were washed and stained using Streptavidin-Phycoerythrin (SAPE). Arrays were processed using the GCOS software and scanned using the Affymetrix Scanner. Expression analysis was performed for all small RNAs for Homo sapiens (e.g., miRNA, small nuclear RNAs like snoRNA and scaRNA) and separately for Sanger miRNA registry content (miRBase 11.0, http://microrna.sanger.ac.uk/) for Homo sapiens.

*2.4. miRNA qRT-PCR.* miRNA reverse transcription was performed using the TaqMan microRNA Reverse transcription Kit (Applied Biosystems) at 16°C for 30 min, 42°C for 30 min, and denaturation of the enzyme at 85°C for 5 min. The RT reaction was performed at 37°C for 1 hour followed by 5 min at 95°C. TaqMan microRNA assays (Applied Biosystems) were performed using the 7500 Fast Real-Time PCR System at the 9600 emulation run mode. Ct values were converted into copy numbers (copy no. = $2^{(-\mathrm{Ct})}$) and normalized to RNU44.

*2.5. Statistical Analysis.* Statistical analyses were performed using GraphPad Prism software. Values are expressed as

means ± SEM. Unpaired Student's $t$-tests were used to compare data. A $P$ value <.05 (two sided) was considered significant. For analysis of microarray expression data, a two-sample $t$-test was carried out for every gene, followed by multiplicity correction to control the false discovery rate (FDR) at .05.

## 3. Results

We initially performed expression profiling of all small RNAs (1770 genes, Figure 1) in the whole blood samples. Three miRNAs passed the FDR cutoff of 0.05: miR-584 (7.9-fold higher in healthy versus CAD patients, $P = .000103$, $t$-test), miR-542-5p (3.9-fold higher in healthy versus CAD pts, $P = .000168$, $t$-test), and miR-187* (2.77-fold higher in healthy versus CAD pts, $P = 8.1 \times 10^{-5}$, $t$-test). However, signal intensities for miR-542-5p and miR-187* were very low and not detected in most samples. Separately, we performed an analysis of Sanger registry miRNAs (848 for Homo sapiens) in the whole blood samples, but none of the miRNAs passed the FDR cutoff of 0.05. Ten miRNAs passed a FDR cutoff of 0.10, but only one (miR-129-5p, 1.57 fold higher in healthy patients, $P = .000044$) of these miRNAs had consistently detectable signal across the 10 blood samples. miR-584 was not among these 10 miRNAs. Although we were able to detect some differences in whole blood miRNA levels between healthy subjects and CAD patients (miR-584, in particular), our microarray data suggest that, similar to other reports [14], levels of miRNAs in the blood are low and microarrays may lack the sensitivity to adequately identify miRNAs that might serve as vascular disease biomarkers. Interestingly, among the miRNAs that tended to show consistent differences between healthy and CAD blood, miRNA expression was generally higher in the blood of healthy subjects, a finding previously observed by others [8].

Next, we performed qRT-PCR on RNA isolated from whole blood of another 10 patients with angiographically significant CAD and 15 healthy subjects. We evaluated the levels of miRNAs that, based on our microarray data, were highly expressed in blood and tended to have different levels of expression between the two groups. This analysis included miR-150, miR-584, miR-21, miR-24, miR-126, miR-92a, miR-34a, miR-19a, miR-145, miR-155, miR-222, miR-378, miR-29a, miR-30e-5p, miR-342, and miR-181d. Among these we found that miR-19a, miR-584, miR-155, miR-222, miR-145, miR-29a, miR-378, miR-342, miR-181d, miR-150, and miR-30e-5p were significantly downregulated in the blood of patients with CAD compared to healthy subjects (Figure 2). Furthermore, we also assessed expression of these 11 miRNAs in a cohort of patients who had ≥2 risk factors for CAD, but did not have angiographically significant CAD. We found that there was no difference in whole blood miRNA expression of this latter group compared to that in patients with significant CAD (not shown), suggesting that these miRNAs are markers for vascular inflammation rather than markers specific for significant CAD.

There has been one report that miRNA expression in blood may be influenced by medications [8]. Indeed, one

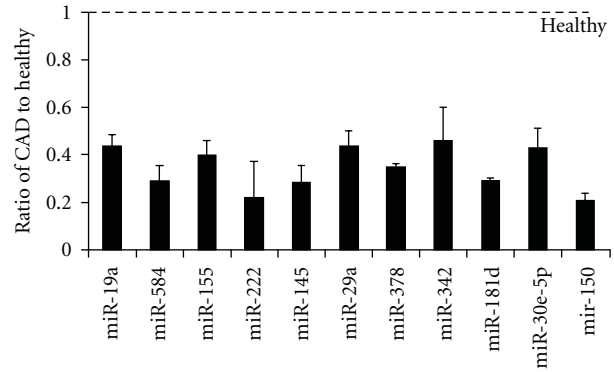


Figure 2: Identification of miRNAs that are decreased in CAD patients compared to healthy subjects. TaqMan miRNA assays were performed on RNA isolated from blood obtained from 2 different groups: patients with angiographically significant CAD ($n = 15$) and healthy volunteers ($n = 5$). $P$ values: miR-19a = .012, miR-584 = .036, miR-155 = .002, miR-222 = .001, miR-145 = .008, miR-29a = .012, miR-378 = .001, miR-342 = .001, miR-30e-5p = .02, and miR-150 = .006.

of the miRNAs that we found to be downregulated in the blood of patients with CAD is miR-155, which is known to target the AT1 receptor [15]. Therefore, we assessed the impact of medications on expression of miR-155, miR-145, miR-181d, miR-222, miR-19a, miR-342, miR-29a, miR-30e-5p, and miR-378. We compared the miRNA expression levels in the blood of patients with angiographically significant CAD or ≥2 risk factors for CAD but not on ACEI or ARB to that in blood of similar patients who were taking at least one of medications. Interestingly, levels of miR-155, miR-19a, miR-145, miR-222, miR-342, miR-30e-5p, and miR-378 (Figure 3) were significantly decreased in patients taking ACEI or ARB compared to those who were not, suggesting that these medications may directly modulate expression of these miRNAs, or they may influence inflammatory factors that otherwise regulate their expression. Importantly, we did not find that statin use had a significant effect on the levels of miRNAs identified in this study (not shown).

## 4. Discussion

Several recent studies have indicated that there is a potential role for circulating miRNA levels as valuable biomarkers for different disease processes, including cancer, cardiomyopathy, and acute myocardial infarction. In this study, we wanted to address the hypothesis that miRNA expression levels in blood could predict the presence of significant coronary artery disease in human subjects. We identified 11 miRNAs whose expression was significantly downregulated in patients with angiographic evidence of significant atherosclerosis compared to healthy subjects that were matched for age and gender. In addition, our data suggest that inhibition of the renin angiotensin system by ACEI or ARB use further influences miRNA expression in blood. This study confirms previous reports showing differences in circulating miRNA levels of patients with CAD compared to those of healthy subjects [8, 16]. Our study differs from the other studies in

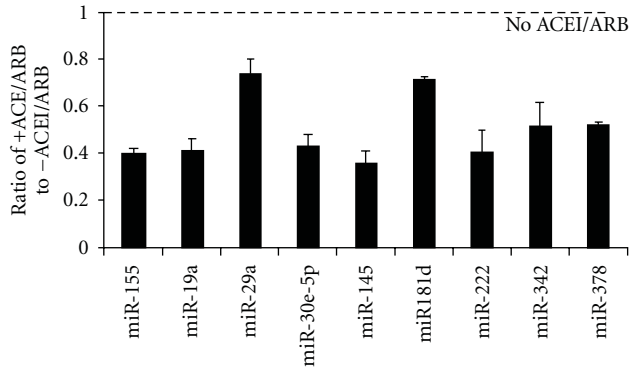


Figure 3: Medications influence the circulating levels of specific miRNAs. Expression data obtained from the patients (angiographically significant CAD or ≥2 CAD risk factors but no significant CAD lesions) on ACEI or ARB compared to similar patients not on ACEI or ARB. Circulating levels of miR-155, miR-19a, miR-378, miR-222, miR-342, miR-145, and miR-30e-5p were decreased in patients who were taking these medications. *P* values: miR-155 = .0007, miR-19a = .035, miR-29a = NS, miR-30e-5p = .011, miR-145 = .003, miR-181d = NS, miR-222 = .002, miR-342 = .0036, and miR-378 = .0004.

the content of the group of miRNAs that were downregulated in patients with CAD.

We performed this analysis using the PAXgene Blood RNA system because it provides a way to stabilize RNA immediately after sample collection and facilitates storage of the samples for a relatively long period of time without compromising RNA integrity [17, 18]. Importantly, mRNA profiling of whole blood or peripheral mononuclear cells has been previously applied to cardiovascular disease [19, 20], and a relationship has been identified between mRNA levels in whole blood and extent of coronary artery disease. Our study extends this work by providing insight into the whole blood expression of miRNAs that are potentially involved in regulating these CAD genes.

In our qRT-PCR analysis of whole blood samples from CAD patients, we found similar changes in expression of miR-155 and miR-145 as what has been reported previously [8]. However, we failed to detect changes in other miRNAs that were described in this previous report, namely miR-126 and miR-92a. There are several possible explanations for this. First, we studied whole blood samples, whereas plasma was studied previously. Thus our miRNA profile likely reflects intracellular and extracellular miRNAs levels in contrast to exclusively extracellular miRNAs that would be detected in plasma. Another difference in our study is that we normalized miRNA expression to expression of RNU44, a small nucleolar RNA that, based on our microarray analysis, we found to be highly and consistently expressed across blood samples from CAD patients and controls.

This study confirmed the observation made by others regarding the difficulty of using microarray analysis to profile miRNA expression in blood samples [14]. Undoubtedly, this is in part due to the limitation of a relatively small sample size for our microarray analysis, as well as reduced sensitivity of the microarray method compared to qRT-PCR. In addition, principal component analysis of our data suggested that two of the miRNA profiles for healthy subjects were actually similar to that of the CAD patients, suggesting that these individuals may in fact have subclinical vascular inflammation. This finding is in the process of being further investigated. Despite this, we believe that careful selection of patients and well-matched control subjects from a larger group of well-characterized individuals reduced some of the variability in our qRT-PCR analysis.

We can only speculate as to why expression of circulated miRNAs is generally decreased in patients with CAD. It has been hypothesized that levels of circulating miRNAs are decreased in vascular disease because they have been taken up into atherosclerotic lesions [8]. The levels of circulating miRNAs may be affected by multiple factors, including transcription, processing and stability of the miRNAs within circulating cells, as well as the ability of these cells to release miRNAs into the plasma. Circulating miRNAs may be delivered to cells in the heart or blood vessels through microvesicles, exosomes, or apoptotic bodies [21]. Because our study assessed miRNA expression in whole blood, our findings are likely more reflective of changes in miRNA transcription or processing rather than release from the circulating cells.

It remains to be determined whether downregulation of miRNAs in CAD patients is directly involved in inflammation or a compensatory response to this process. Based on our observed changes in miRNA expression in response to ACEI/ARB therapy, we believe that circulating miRNA levels reflect a compensatory response to inflammation. Further experimental studies are necessary to explore the mechanisms by which CAD and therapies affect tissue versus circulating miRNA levels.

In summary, the present study provides insight into whole blood levels of miRNAs in patients with CAD compared to healthy subjects and demonstrates their potential utility as biomarkers for vascular disease. Validation of the changes in miRNA expression observed here in larger studies will be a necessary step to confirm their candidacy as biomarkers and therapeutic targets. We believe that further elucidation of the role that these miRNAs play in the pathogenesis and progression of chronic CAD will contribute to our understanding of the disease process and lead to new therapeutic and preventative strategies.

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*Review Article*

# Peripheral Vascular Dysfunction in Chronic Kidney Disease

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There is an increased prevalence of cardiovascular disease- (CVD-) related mortality in patients with chronic kidney disease (CKD). Endothelial dysfunction is a primary event in the development of atherosclerosis and hypertension and likely contributes to the elevated cardiovascular risk in CKD. Endothelial dysfunction has been shown to occur in the peripheral vasculature of patients with both severe and moderate CKD. Mechanisms include oxidative stress, L-arginine deficiency, and elevated plasma levels of ADMA. Interventions designed to restore vascular function in patients with CKD have shown mixed results. Evidence from cell culture studies suggest that the accumulation of uremic toxins inhibits L-arginine transport and reduces nitric oxide production. The results of these studies suggest that endothelial dysfunction may become less reversible with advancing kidney disease. The purpose of this paper is to present the current literature pertaining to potential mechanisms of peripheral vascular dysfunction in chronic kidney disease and to identify possible targets for treatment.

## 1. Introduction

Chronic kidney disease (CKD) is a major public health concern affecting nearly 20 million people in the United States alone [1]. Patients with CKD are at greater risk of cardiovascular disease- (CVD-) related morbidity and mortality than individuals without CKD with similar cardiovascular risk factors and tend to die before reaching end-stage renal disease (ESRD) [2–5]. Treatments aimed solely at reducing traditional cardiovascular risk factors do not improve cardiovascular function in patients with late-stage CKD [6]. Therefore, traditional CVD risk factors alone cannot explain the high incidence of CVD in CKD.

Endothelial dysfunction is a precursor to the development of atherosclerosis [7, 8] and has been shown to be associated with increased cardiovascular risk in patients with left ventricular dysfunction and congestive heart failure [9–11]. The combination of traditional risk factors in CKD is not enough to explain the high incidence of CVD and endothelial dysfunction has been suggested to play a role in the increased CV risk in CKD [12, 13]. In support of this, a longitudinal study of patients with end-stage renal disease (ESRD) found all cause mortality to be independently associated with impaired endothelial function [14].

The majority of studies of endothelial function in renal disease have focused on ESRD and patients receiving dialysis; however, little is known about endothelial function in earlier stages of CKD. Guidelines from the Kidney Disease Outcomes Quality Initiative (K/DOQI) place patients with CKD into one of five stages based on glomerular filtration rate (GFR) and are presented in Table 1 [1, 15]. The risk of developing CVD can be predicted from GFR and increases as GFR declines [3]. These guidelines make it easier to delineate differences in vascular function in CKD patients with low to moderate renal deficiency; however to date those data are limited. With the majority of patients dying of cardiovascular disease before needing dialysis, there is an urgent need for investigations into vascular function in earlier stages of CKD. A great deal of literature has described the role of vascular calcification and aortic stiffness in promoting atherosclerosis in CKD and have been reviewed elsewhere [16]. The purpose of this paper is to present a summary of the available research regarding mechanisms of peripheral vascular dysfunction throughout the progression of chronic kidney disease.

Table 1: Stages of CKD.

| Stage | Description | GFR, mL·min$^{-1}$ per 1.73 m$^2$ |
|---|---|---|
| 1 | Kidney damage with normal or increased GFR | ≥90 |
| 2 | Kidney damage with mildly decreased GFR | 60–89 |
| 3 | Moderately decreased GFR | 30–59 |
| 4 | Severely decreased GFR | 15–29 |
| 5 | Kidney failure | <15 or dialysis |

Adapted from National Kidney Foundation K/DOQI clinical practice guidelines for chronic kidney disease [15].

## 2. Vascular Function in CKD

The vascular endothelium consists of a single layer of cells lining the interior lumen of blood vessels. Aside from functioning as a barrier between the intravascular space and the interstitum, the vascular endothelium works as an important regulator of vascular homeostasis. Perhaps one of the most important functions of the endothelium is the synthesis and release of the vasodilator nitric oxide [17]. Nitric oxide is produced from the amino acid substrate L-arginine by the enzyme endothelial nitric oxide synthase (eNOS) [18]. Activation of eNOS requires the binding of the cofactor tetrahydrobiopterin ($BH_4$) as well as the presence of $Ca^{2+}$/Calmodulin for the synthesis of NO [19]. NO diffuses through the basal membrane of the endothelial cell into the smooth muscle where it activates the conversion of GTP to cyclic GMP by soluble guanylyl cyclase (sGC) signaling smooth muscle relaxation and vasodilation [20]. This pathway occurs in response to mechanical shear stress or the binding of bradykinin or acetylcholine to their receptors on endothelial cells and is vital for proper control and maintenance of blood pressure. Additionally, nitric oxide plays an important role by regulating vascular permeability, leukocyte adhesion [21], and smooth muscle proliferation [22], all of which play an important role in maintaining cardiovascular health [23]. Endothelial dysfunction is characterized by a reduced synthesis or bioavailability of NO and is a primary event in the development of atherosclerosis [24]. *In vivo* assessment of conduit artery and microvascular function through flow-mediated dilation (FMD) and venous occlusion plethysmography (VOP) have been used to assess vascular function in CKD (Table 2) and serve as measures of endothelial function.

Endothelial dysfunction is present in CKD and may explain the increased cardiovascular risk in these patients. Urinary nitrate and nitrite (NOx) excretion, an index of total body NO production, is lower in patients with ESRD and likely contributes to the hypertension observed in these patients [36]. Endothelial dysfunction has been shown to occur *in vitro* in human microvessels obtained via subcutaneous fat biopsies from patients receiving dialysis or renal replacement surgery [37] as well as *in vivo* in the forearm microvasculature of predialysis CKD patients using venous occlusion plethysmography [28]. Further, endothelial dysfunction has been shown to occur in conduit vessels upstream of the microvasculature [27, 34]. Thus, endothelial dysfunction appears to be characteristic of CKD.

The confounding effects of other risk factors in CKD make it difficult to differentiate whether the source of endothelial dysfunction is due to renal impairment or the combination of multiple risk factors. Studies using children with CKD have assessed endothelial function before atherosclerosis and other associated risk factors were believed to occur [38–40]. Endothelial-dependent dilation was shown to be impaired in children with chronic renal failure using flow-mediated dilation (FMD) suggesting that kidney disease alone leads to the development of endothelial dysfunction [38]. Additionally, nitric oxide plays an important role in attenuating renal impairment and may slow the progression of CKD in patients with mild renal impairment. Reduced NO bioavailability has been shown to contribute to glomerular hypertension and accelerate renal damage leading to more rapid progression of CKD [41]. While the presence of endothelial dysfunction is well documented in CKD, the mechanisms have not yet been fully explained.

## 3. Potential Mechanisms of Endothelial Dysfunction in CKD

*3.1. Oxidative Stress.* One of the most widely studied contributors to endothelial dysfunction is oxidative stress [42]. Oxidative stress is defined by a disruption in the balance between free radical production and removal by endogenous antioxidants [43]. Oxidative stress impairs NO signaling pathways in endothelial cells and contributes to endothelial dysfunction [43]. Oxidative stress is present in patients with moderate to severe CKD [32, 44] as well as patients with ESRD or receiving hemodialysis [32, 45]. Endothelial dysfunction worsens with progressive loss of renal function and is associated with a variety of markers of oxidative stress including lipid hydroperoxides, oxidized glutathione, protein carbonyls and F2-isoprostanes [29, 31, 44, 45] as well as reduced antioxidant capacity [46, 47].

Markers of oxidative stress are inversely correlated with endothelial function in patients with CKD. In one study, brachial-artery flow-mediated dilation was decreased in hemodialysis patients and correlated to increased levels of TBARS, a marker of lipid peroxidation [33]. Interestingly, these patients lacked overt symptoms of cardiovascular disease suggesting that oxidative stress may contribute to the early progression of endothelial dysfunction in CKD. Endothelial function and GFR have also been shown to be associated with increased levels of advanced glycation end products (AGEs) in different stages of predialysis, nondiabetic CKD, likely through increased abundance of AGE receptors (RAGE) on the surface of endothelial cells [48]. AGEs have been shown to increase inflammation and oxidative stress [48] and can also inhibit eNOS activity directly as well as reduce substrate bioavailability by reacting with L-arginine resulting in endothelial dysfunction [49].

The mechanisms behind the contribution of oxidative stress in endothelial dysfunction in CKD appear to occur primarily through eNOS and NO dependent pathways

Table 2: Summary of studies measuring *in vivo* endothelial function in adult patients with CKD.

| Study | Reported degree of CKD | Method | Finding |
|---|---|---|---|
| Yilmaz et al. [25] | Stage 1 | FMD | Improved following 12-week treatment with ramipril |
| Yilmaz et al. [26] | Stage 1–4 | FMD | Improved following 3-month treatment with ramipril or valsartan |
| Nanayakkara et al. [27] | CCr = 38 ± 15 | FMD | Improvement following 18-month stepwise treatment with pravastatin, vitamin E, and homocysteinie lowering therapy |
| Annuk et al. [28] | CCr = 29.4 ± 24.0 | VOP | Impaired and related to degree of renal impairment |
| Annuk et al. [29] | CCr = 25.1 ± 16.2 | VOP | Impairments correlated to markers of oxidative stress |
| Annuk et al. [30] | Serum Cr = 287 ± 143 | VOP | Improved by acute COX inhibition or L-arginine treatment |
| Annuk et al. [31] | Stage 3–5 | VOP | Negatively correlated to levels of lipid hydroperoxides (LOOH) |
| Ghiadoni et al. [32] | Stage 3–5, hemodialysis | FMD | Acute vitamin C infusion restored impaired function in hemodialysis but not in CKD |
| Costa-Hong et al. [33] | ESRD | FMD | Plasma TBARS levels associated with impaired endothelial-dependent dilation in patients without symptoms of CVD |
| Cross et al. [34] | Predialysis hemodialysis | VOP, FMD | Acute infusion of vitamin C improves endothelium-dependent dilation in forearm resistance vasculature but not in brachial artery |
| Cross et al. [35] | Predialysis, hemodialysis | VOP, FMD | Local or systemic L-arginine infusion did not improve resistance or endothelial-dependent dilation |

FMD = flow-mediated dilation; VOP = venous occlusion plethysmography; CCr = creatinine clearance (mL/min/1.73 $m^2$); Serum Cr = serum creatinine ($\mu$mol/L).

(Figure 1). Cross et al. [34] observed a reduction in acetylcholine-induced forearm blood flow (venous occlusion plethysmography) in predialysis patients with ESRD (GFR <20 mLmin). This impairment was ameliorated when the antioxidant vitamin C was infused through the brachial artery suggesting that the source of endothelial dysfunction was oxidative stress. This improvement was abolished when vitamin C was infused with the NOS inhibitor L-NMMA suggesting that the effect of oxidative stress on forearm blood flow is NO dependent. Interestingly, vitamin C did not cause an improvement in endothelial function in larger upstream conduit vessels as assessed by brachial and radial artery flow-mediated dilation in both predialysis and dialysis patients with severe renal failure [34]. This finding suggests that some other mechanism contributes to endothelial dysfunction in late-stage kidney disease.

Although oxidative stress has been shown to be more severe in hemodialysis patients than in patients with stage 3–5 CKD [32], studies have shown oxidative stress to contribute to endothelial dysfunction earlier in CKD. Perfused mesenteric arteries from rats that underwent renal mass reduction have impaired vasodilation in response to acetylcholine within 3–10 days after surgery [50]. This impairment was restored by incubation with the antioxidant enzyme superoxide dismutase (SOD) [50] suggesting that oxidative stress also plays a role in endothelial dysfunction early in the progression of CKD. In human patients with moderate renal impairment, the forearm blood flow response to an arterial infusion of methacholine was attenuated [29]. These same patients also demonstrated increased levels of lipid hydroperoxides (LOOH) and an increased ratio of oxidized to reduced glutathione (GSSG/GSH). These markers of oxidative stress were correlated to both serum creatinine and forearm blood flow suggesting that oxidative stress contributes to endothelial dysfunction in humans with moderate CKD.

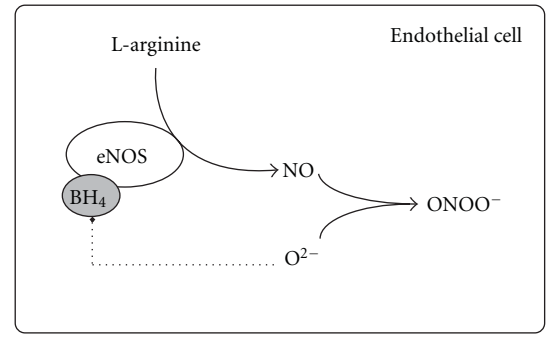


Figure 1: Simplified schematic depicting mechanisms by which reactive oxygen species can reduce nitric oxide (NO) availability. NO is synthesized from eNOS and can combine with excess superoxide ($O^{2-}$) from vascular oxidases to form peroxynitrite ($ONOO^-$) limiting NO availability. Superoxide can also oxidize the eNOS cofactor tetrahydrobiopterin ($BH_4$), uncoupling eNOS and reducing NO synthesis.

Supplementation with antioxidants has been shown to reduce cardiovascular events in CKD and hemodialysis [51]. In the Secondary Prevention with Antioxidants of Cardiovascular Disease in Endstage Renal Disease (SPACE) trial, hemodialysis patients receiving 800 IU/day of vitamin E had significantly fewer primary cardiovascular end-points than placebo-treated controls [52]. The benefits of antioxidant therapy have also been studied in earlier stages of CKD and have been shown to improve endothelial function. The Antioxidant Therapy in Chronic Renal Insufficiency (ATIC)

study demonstrated that antioxidant treatment in patients with mild to moderate renal impairment was effective at improving brachial artery flow-mediated dilation [27]. Patients underwent three stages of drug treatment consisting of treatment with pravastatin, vitamin E, and homocysteine-lowering therapy introduced consecutively at 6 month intervals. Additionally, this improvement in endothelial function occurred despite a progressive loss in renal function. Antioxidant treatment has not been effective at reducing cardiovascular risk in other diseases [53]; however, these studies suggest that the use of antioxidants in CKD may be an exception.

Another strategy for combating oxidative stress mediated endothelial dysfunction may be bolstering endogenous antioxidant defense mechanisms. Endogenous antioxidant enzymes play an important role in preventing oxidative stress and are impaired in CKD. Plasma and erythrocyte levels of glutathione peroxidase (GSH-px), superoxide dismutase (SOD), and catalase were shown to be significantly lower in predialysis patients with ESRD compared to healthy control subjects [46]. Endogenous antioxidants have also been shown to be lower in earlier stages of CKD with levels of erythrocytic GSH-px, and SOD impaired as early as stage 1 (GFR <90 mL/min) [45]. Treatments aimed at improving the endogenous antioxidant defense systems may be more effective than antioxidant therapy. Chronic exercise training was shown to reduce levels of ROS and improve endothelial-dependent relaxation in 5/6 nephrectomized rats [54]. Exercise has been shown to improve endothelial-dependent dilation in aging [55] and may help prevent the progression of endothelial dysfunction in CKD; however, further study is needed.

The role of oxidative stress on vascular function in CKD is well documented however more research is needed to uncover the sources and specific targets of free radicals. The production of the powerful radical superoxide from NADPH oxidases is a likely contributor oxidative stress in the endothelium [43] and can combine with NO to form reactive nitrogen species such as peroxynitrite ($ONOO^-$) (Figure 1). Peroxinitrite formation reduces the bioavailibilty of NO and contributes to nitrositive cellular damage. Vaziri et al. [56] measured increased nitrotyrosine abundance, a marker of nitrositive protein modification, in aorta, heart, liver, and plasma of 5/6 nephrectomized rats. Nitrotyrosine levels were decreased to normal levels in rats treated with the antioxidant vitamin E. Angiotensin II is elevated in CKD and has been shown to activate NADPH oxidases [57]. Few studies have examined the role of inhibition of the renin-angiotensin system on endothelial-dependent dilation in CKD. One study observed improved FMD following short-term ACE-inhibition in patients with stage 1 diabetic CKD [25]. Additional free radical production may occur from xanthine oxidase; however, the role of this enzyme in humans with CKD is not well known. Treatment with the xanthine oxidase inhibitor allopurinol slowed the progression of CKD, and reduced cardiovascular risk in patients with CKD (eGFR <60 mL/min) [58]; however, it is unclear if these beneficial effects were due to decreased oxidative stress or reduced serum uric acid levels.

Oxidation of the essential eNOS cofactor tetrahydrobiopterin ($BH_4$) has been shown to cause uncoupling of eNOS (Figure 1). Once uncoupled, eNOS itself becomes a source of superoxide contributing to further oxidative damage [59, 60]. Chronic treatment with $BH_4$ has been shown to lower systolic blood pressure and reduce proteinuria in 5/6 nephrectomized rats [61]. In another study, endothelial-dependent relaxation of aortic rings from 5/6 nephrectomized rats was restored in isolated vessels treated with $BH_4$ [62]. These vessels were also associated with increased superoxide production which was ameliorated by treatment with L-NAME suggesting that the source of oxidative stress was uncoupled eNOS. The presence of oxidative stress in CKD is well defined and is a contributing factor to the development of endothelial dysfunction. Treatments aimed at restoring vascular redox balance may be effective at reducing cardiovascular risk in CKD. In addition, supplementation with $BH_4$ in humans with CKD may prevent eNOS uncoupling and reduce oxidative stress.

*3.2. L-Arginine Deficiency.* Nitric oxide synthesis relies on the amino acid substrate L-arginine. L-arginine synthesis primarily occurs in the proximal tubules of the renal cortex and is impaired with loss of functional renal mass [41]. Despite reduced production, the plasma concentration of L-arginine in patients with CKD appears to be maintained at normal levels [63]. The maintenance of plasma L-arginine levels in CKD may be a consequence of increased amino acid release into the blood due to skeletal muscle wasting [64], and impaired L-arginine transport due to increased uremic toxins [65, 66]. Both mechanisms could potentially mask the decrease in L-arginine production by maintaining the plasma concentrations.

The intracellular concentration of L-arginine in endothelial cells is normally much higher than the Km of eNOS for L-arginine [67]. Despite the apparent cellular abundance of substrate, exogenous treatment with L-arginine has been effective at restoring endothelial function in other disease states including aging [68] and hypercholesterolemia [69, 70]. Cooke et al. [69] demonstrated that endothelial-dependent relaxation of aortic rings was restored in hypercholesterolemic rabbits pretreated with an intravenous infusion of L-arginine. In 5/6 nephrectomized rats, both chronic and acute treatment with L-arginine improved blood pressure and restored endothelial-dependent relaxation of aortic rings [62]. This phenomenon, referred to as the "arginine paradox" [67, 71], was not observed, however, in patients receiving dialysis or in predialysis patients with severe renal failure [35]. In another study, infusion of L-arginine into the forearm improved the endothelial-dependent dilatory response to methacholine in both patients with CKD and aged-matched controls [30]. Age may have been a confounding factor in this study, however, since subjects were between 50 and 80 years of age.

L-arginine transport has been shown to be impaired by uremic toxins and may potentially explain why L-arginine treatment has been ineffective in later stages of CKD (Figure 2). Endothelial cells cultured in uremic plasma had a reduced ability to transport L-arginine into the cells [65].

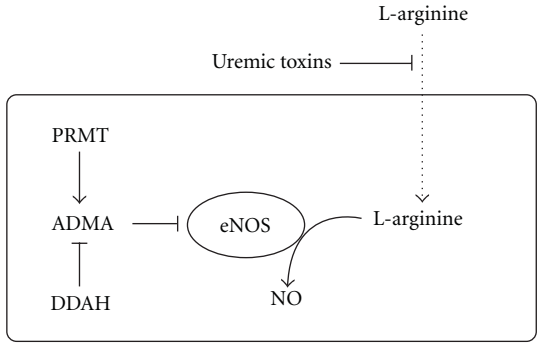


Figure 2: Potential mechanisms of L-arginine deficiency in chronic kidney disease (CKD). Asymmetric dimethylarginine (ADMA) is a competitive inhibitor of eNOS and ADMA levels are increased in CKD. Synthesis of ADMA occurs via protein arginine methyltransferase (PRMT) which may exhibit increased expression and activity in CKD. Expression of the enzyme dimethylarginine dimethylaminohydrolase (DDAH) may be decreased in CKD resulting in reduced degradation of ADMA. L-arginine transport into endothelial cells can be inhibited by increased levels of uremic toxins as disease progresses and result in reduced substrate availability for NO production.

When the same experiment was repeated with cells cultured in a synthetic solution containing uremic levels of urea, the same result was observed indicating that urea is an important inhibitor of L-arginine transport. The transport of L-arginine occurs through the cationic amino acid transporter CAT-1. Urea transport occurs through urea transporters (UT) that appear to be colocalized with CAT-1 in endothelial cells [66]. The movement of urea into the cell appears to be required in order to inhibit L-arginine transport. This mechanism may occur with the accumulation of uremic toxins as seen in CKD and lead to impaired L-arginine transport and reduced substrate available for NO synthesis.

The uremic inhibition of L-arginine transport appears to occur in an "all-or-nothing" fashion [41, 65]. Inhibition of L-arginine transport was only effective with a synthetic solution of urea at or above 15 mmol/L and not effective at lower concentrations [65]. Interestingly, when uremic plasma was diluted to achieve a lower urea concentration below this threshold, L-arginine transport was still inhibited. This suggests that other uremic toxins also contribute to transport inhibition and should be studied more extensively in order to elucidate their mechanisms.

Hyperhomocysteinemia reduced L-arginine uptake in cultured endothelial cells and impaired endothelial-dependent relaxation in homocysteine-treated vessels and was associated with oxidative stress and increased nitrotyrosine levels [72]. Treatment with vitamin C restored cellular uptake of L-arginine while L-arginine treatment improved endothelial-dependent relaxation of aortic rings. CAT-1 expression was also attenuated in homocysteine-treated cells and was related to impaired NO production with no change in eNOS expression or activity. Hyperhomocysteinemia is present in patients with CKD [4] and may contribute to the uremic inhibition of L-arginine transport in humans; however, this has not been studied.

The accumulation of uremic toxins in the plasma occurs progressively with loss of renal function. Increased levels of urea in humans has been correlated to decreased endothelial-dependent dilation [31] and may be related to reduced L-arginine transport. While L-arginine treatment has been ineffective at later stages of CKD and during hemodialysis, it is plausible that treatment in earlier stages, when the influence of uremic toxins is not as robust, may be effective at restoring endothelial function.

*3.3. ADMA in CKD.* Another potential contributor to endothelial dysfunction in CKD is the formation of the endogenous NOS inhibitors asymmetric dimethylarginine (ADMA) and *N*-monomethylarginine (L-NMMA) [73, 74]. ADMA and L-NMMA are the result of posttranscriptional methylation of L-arginine residues by protein arginine methyltransferases (PRMTs) and are released in their free form following protein hydrolysis. ADMA production is about 10-fold that of L-NMMA and is elevated in patients with chronic renal failure [75]. Plasma levels of ADMA predict progression to ESRD in patients with CKD [76] and ultimately predict adverse cardiovascular events in patients with mild to moderate CKD [77, 78]. ADMA is associated with impaired endothelial function in healthy adults [79], and has been shown to be inversely correlated to brachial artery FMD in patients with proteinuria and amyloidosis [26].

ADMA has been classified as a "uremic toxin" and exhibits adverse cardiovascular effects [4]. In healthy subjects infused with ADMA, heart rate and cardiac output were reduced while mean arterial pressure increased [80]. ADMA has also been linked to impaired endothelial function. Brachial artery FMD was impaired in healthy individuals treated with L-NMMA suggesting that a reduction in the ratio or L-arginine to methylated arginines competitively inhibits NO production *in vivo* [35]. The addition of L-arginine in these patients improved this balance and restored endothelial function. While L-arginine treatment is an effective strategy in restoring substrate balance and combating ADMA and L-NMMA in other disease models, its effectiveness in CKD is questionable due to the L-arginine transport issues discussed above. It is therefore important to explore alternative strategies for reducing circulating levels of ADMA in kidney disease.

Interventions designed to lower ADMA production or increase ADMA clearance may be effective at improving endothelial function in CKD. Clearance of ADMA in the urine is impaired with renal damage and contributes to elevated plasma concentrations [81]; however, this is not the primary reason for elevated ADMA in CKD. Urinary clearance of ADMA did not explain elevations in plasma ADMA in an animal model of CKD [82]. Instead, increased PRMT activity and expression and reduced degradation of ADMA by dimethylarginine dimethylaminohydrolase (DDAH) are likely the major causes of increased ADMA in CKD [41] (Figure 2). PRMT expression and activity is

increased in the presence of oxidized LDL cholesterol and results in increased production of ADMA in endothelial cells [83]. Antioxidant therapy with vitamin E has been shown to reduce ADMA levels in patients with CKD [84] and may be an effective treatment strategy to restore endothelial function. PRMT expression was increased in an animal model of CKD [82] and may be a potential therapeutic target to restore endothelial function.

DDAH converts ADMA to dimethylarginine and L-citrulline and has been shown to decrease expression in an animal model of CKD [82]. A useful method for studying the role of ADMA in disease states is through the modification of DDAH enzyme expression in animal models. In mice infected with recombinant DDAH, NO synthesis was significantly increased while plasma ADMA concentrations were reduced compared to wild-type animals [85]. Endothelial-dependent relaxation was impaired in streptozotocin-induced diabetic rats and restored by overexpression DDAH [86]. When isolated mouse carotid artery sections were transfected with recombinant DDAH in the presence of exogenous ADMA, endothelial-dependent relaxation was improved compared to uninfected vessels [87]. ACE inhibition may also reduce ADMA levels and contribute to improved endothelial function. One study showed that 3 months of treatment with the ACE inhibitor Ramipril (5 mg/day) or the angiotensin II receptor antagonist Valsartan (160 mg/day) reduced ADMA levels and restored FMD in patients with nondiabetic CKD [26].

## 4. Conclusion

The endothelium represents the largest organ in the human body and is vital for the maintenance of vascular homeostasis. There is an urgent need to understand the mechanisms by which the endothelium becomes impaired in CKD in order to design more effective strategies for reducing cardiovascular risk. Chronic kidney disease is a worldwide health concern and leads to an accelerated risk of cardiovascular death. Because there are a significantly lower number of patients with later stages of CKD likely due to CVD-related death [3], earlier stages of CKD should be more extensively studied in order to better predict and prevent cardiovascular mortality in CKD. There is limited but convincing data supporting the role of endothelial dysfunction in earlier stages of CKD. Future research should focus on better understanding the mechanisms of endothelial dysfunction as well as development of effective interventions in earlier stages of CKD where the risk of cardiovascular death is most evident.

Evidence suggests that oxidative stress, L-arginine deficiency, and ADMA inhibition of NO synthesis all play roles in the pathogenesis of endothelial dysfunction in CKD. Based upon differences between patients with ESRD and earlier stages of CKD, it appears that there may be a point at which endothelial dysfunction becomes less reversible with advancing kidney disease [27]. Evidence from cell culture studies linking the impaired transport of L-arginine to uremic toxins supports this hypothesis and may be applied to other mechanisms of endothelial dysfunction. The increased uremic burden that accompanies late-stage CKD may trigger a "uremic switch" resulting in endothelial dysfunction that is less reversible due to an inability to transport L-arginine into endothelial cells. Once triggered, conventional therapies for treating endothelial dysfunction may become less effective leading to the increased prevalence of CVD seen in late-stage CKD. It is therefore necessary to understand the pathogenesis of endothelial dysfunction in earlier stages of CKD in order to prevent the deterioration of vascular function.

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*Review Article*

# Endothelium-Derived Hyperpolarizing Factor and Vascular Function

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Endothelial function refers to a multitude of physiological processes that maintain healthy homeostasis of the vascular wall. Exposure of the endothelium to cardiac risk factors results in endothelial dysfunction and is associated with an alteration in the balance of vasoactive substances produced by endothelial cells. These include a reduction in nitric oxide (NO), an increase in generation of potential vasoconstrictor substances and a potential compensatory increase in other mediators of vasodilation. The latter has been surmised from data demonstrating persistent endothelium-dependent vasodilatation despite complete inhibition of NO and prostaglandins. This remaining non-NO, non-prostaglandin mediated endothelium-dependent vasodilator response has been attributed to endothelium-derived hyperpolarizing factor/s (EDHF). Endothelial hyperpolarization is likely due to several factors that appear to be site and species specific. Experimental studies suggest that the contribution of the EDHFs increase as the vessel size decreases, with a predominance of EDHF activity in the resistance vessels, and a compensatory up-regulation of hyperpolarization in states characterized by reduced NO availability. Since endothelial dysfunction is a precursor for atherosclerosis development and its magnitude is a reflection of future risk, then the mechanisms underlying endothelial dysfunction need to be fully understood, so that adequate therapeutic interventions can be designed.

## 1. Introduction

Endothelial function refers to a multitude of physiological processes of the vascular endothelium that maintain healthy homeostasis of the vascular wall and may be used as a "barometer" of the injury/repair inflicted by multiple environmental and genetic factors [1–3]. Vascular endothelial dysfunction is associated with a reduction in nitric oxide (NO) bioavailability, an increase in generation of potential vasoconstrictor substances such as superoxide anions and endothelin–1, and a potential compensatory increase in other mediators of vasodilation. This remaining non-NO, non-prostaglandin-mediated endothelium-dependent vasodilation has been partly attributed to endothelium-derived hyperpolarizing factor/s (EDHF). Endothelial hyperpolarization is likely due to several factors that are site- and species-specific, ultimately causing vascular smooth muscle hyperpolarization and relaxation. Experimental studies suggest that the contribution of EDHFs increase as the vessel size decreases, with predominant EDHF activity in the resistance vessels and a compensatory upregulation of EDHFs in states characterized by reduced NO availability [4–12]. Whereas prostacyclin and NO bioavailability have been extensively investigated in the human circulation *in vivo*, little is known about endothelial hyperpolarization.

## 2. Endothelium-Derived Hyperpolarizing Factor (EDHF)

Potential EDHFs differ by species and vascular bed, but act by increasing potassium ($K^+$) conductance resulting in the subsequent propagation of depolarization of vascular smooth muscle cells and relaxation [13–15] (Figure 1). Acetylcholine causes hyperpolarization of vascular smooth muscle in arteries with an intact endothelium but not in its absence [16–19]. This hyperpolarization is mimicked by

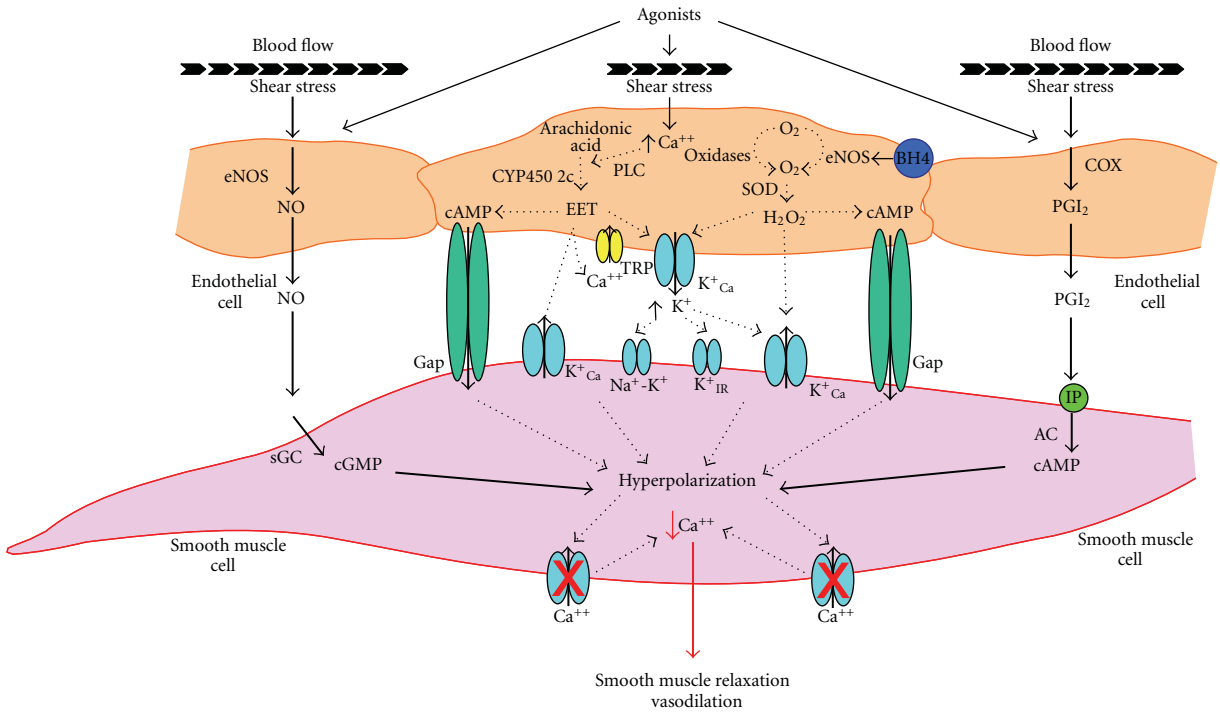


FIGURE 1: Mechanisms for endothelial cell mediated relaxation. Agonist (bradykinin/acetylcholine/substance P) or shear stress increases the activity of endothelial NO synthase (eNOS) and cyclooxygenase (COX), providing nitric oxide x(NO) and prostacyclin($PGI_2$)-mediated dilation. There are multiple potential EDHF pathways. Increases in intracellular calcium activates phospholipase A2 (PLC) to produce arachidonic acid. Its metabolism by cytochrome P450 2C (CYP4502c) generates eicosatrienoic acids (EETs) that can stimulate calcium dependent potassium ($K_{Ca}^+$) channels in endothelial and smooth muscle cells. EETs may also directly activate gap junctions (Gap). EETs may also act in an autocrine manner on endothelial cells by activating transient receptor potential (TRP) V4 channels, which promote Calcium ($Ca^{++}$) influx further increasing the calcium concentration and activating $K_{Ca}^+$ channels to cause hyperpolarization and release of $K^+$ ions into the subendothelial space. The increase in potassium in the interstitium may activate $K_{Ca}^+$ channels, inwardly rectifying potassium channels ($K_{IR}^+$), or the $Na^+$-$K^+$ pump on smooth muscle cells and cause hyperpolarization. Smooth muscle hyperpolarization in turn results in relaxation by closing voltage-gated channels leading to a fall in $Ca^{++}$ concentration and subsequent vasodilation. The action of eNOS (with cofactor tetrahydrobiopterin [BH4]) and oxidases on oxygen ($O_2$) produces the reactive oxygen species superoxide ($\cdot O_2-$). Hydrogen peroxide ($H_2O_2$) generated by dismutation of superoxide anions by superoxide dismutase (SOD) can also cause hyperpolarization by activating endothelial and smooth muscle $K_{Ca}^+$ channels or by gap junctions. Adenylyl cyclase: AC; cyclic Adenosine monophosphate: cAMP; cyclic guanosine monophosphate: cGMP; soluble guanylyl cyclase: sGC; prostacyclin receptor, IP.

certain $K^+$ channel agonists and is unaffected by inhibitors of nitric oxide synthase or cyclooxygenase and has been attributed to release of EDHF/s. EDHFs appear to open smooth muscle cell $K^+$ channels, allowing $K^+$ efflux along its chemical gradient resulting in membrane hyperpolarization. Thus, EDHF activity may be defined functionally as agonist-induced, endothelium-dependent relaxation that is not blocked by inhibitors of NO synthase or cyclooxygenase but can be inhibited, at least in part, by $K^+$ channel blockers.

### 2.1. Potassium ($K_{Ca}^+$) Channel Activation (Figure 1)

*2.1.1. Calcium-Activated Potassium ($K_{Ca}^+$) Channels.* Agonists such as bradykinin stimulate endothelial G protein-coupled receptors provoking an increase in intracellular calcium $[Ca_{2+}]_i$ in the endothelial cell [20, 21]. Endothelium-dependent hyperpolarization of smooth muscle cells results from the opening of $K^+$ channels in the smooth muscle plasmalemma and is abolished by $K^+$ concentrations higher than 25 mM [22]. Agonists that produce hyperpolarization also stimulate efflux of $K^+$ [17, 23]. However, there are several smooth muscle $K^+$ channels; endothelium-dependent hyperpolarization is not prevented by glibenclamide, an inhibitor of ATP-dependent $K^+$ channels ($K_{ATP}^+$), or inhibitors of inwardly rectifying potassium channels ($K_{IR}^+$) [24]. However, both barium chloride (<100 $\mu$mol/L) and ouabain (2.7 nmol/min), inhibitors of $K_{IR}^+$ channels, reduced resting flow in healthy subjects, an effect that was lower in obesity, even though these are not endothelium-dependent responses [25].

The hallmark of the EDHF-mediated responses is its abolition by the combination of apamin (a specific inhibitor of $K_{Ca}^+$ channels of small conductance ($SK_{Ca}^+$ channels)) plus charybdotoxin (a nonselective inhibitor of large-conductance ($BK_{Ca}^+$) and intermediate-conductance ($IK_{Ca}^+$) channels), and of some voltage-dependent $K^+$($K_V^+$) channels [26–28]. This toxin combination targets $K_{Ca}^+$ channels on endothelial cells rather than $K^+$ channels located on smooth

muscle cells. Increasing intracellular free calcium in endothelial cells opens $K_{Ca}^+$ channels allowing efflux and accumulation of $K^+$ into the myoendothelial space. This triggers several processes that explain the EDHF phenomena; including (1) synthesis of cytochrome that P450 (CYP450) metabolites, a family of epoxides, (2) transmission of endothelial cell hyperpolarization to the vascular smooth muscle via gap junctions, and (3) $K^+$ released from the endothelial cells via $K_{Ca}^+$ channels induces smooth muscle hyperpolarization by activating $K_{Ca}^+$ channels and/or $Na^+$-$K^+$-ATPase on vascular smooth muscle cells.

Study of gastroepiploic conductance arteries and microvessels revealed that the contribution of EDHF to endothelium-dependent relaxations is significantly larger in human microvessels than in large arteries, that EDHF-mediated relaxations are mediated via activation of $K^+$ channels, and that risk factors, particularly hypercholesterolemia and aging, were associated with impaired EDHF-mediated dilation [4]. Convincing evidence has been presented to suggest that a CYP450-dependent EDHF plays a significant role in the regulation of coronary arteriolar tone by $K_{Ca}^+$ channel activation and smooth muscle hyperpolarization [8, 29]. Thus, L-NG-nitro arginine/indomethacin-insensitive component of acetylcholine-mediated relaxation was sensitive to 25 mmol/L $K^+$, but not to glibenclamide, a $K_{ATP}$ channel inhibitor. Importantly, relaxation in human subcutaneous resistance arteries was abolished by a combination of charybdotoxin and apamin indicating the crucial role for endothelial $K_{Ca}^+$ channel activation [7].

Human studies have used tetraethylammonium chloride (TEA) to investigate the role of large $K_{Ca}^+$ channel activation on forearm blood flow and on the vascular effects of bradykinin. TEA selectively blocks $K_{Ca}^+$ channels in arterial smooth muscle cells at concentrations of >1 mmol/L [29]. At these doses, TEA decreased resting forearm blood flow by 23% and radial artery diameter by 5%, and it also inhibits bradykinin- and substance P-induced, but not acetylcholine-mediated vasodilation after inhibition of NO and prostaglandins in the forearm microcirculation [30–32]. Moreover, resting radial arterial blood flow and diameter were reduced only with combined blockade of NO synthesis and $K_{Ca}^+$ channels but not with either blockade individually, suggesting an important interaction between these two vasodilator systems [31]. These observations demonstrate the contribution of both NO and $K_{Ca}^+$ channel activation to resting conductance artery and microvascular tone in the healthy human forearm circulation.

*2.2. Epoxyeicosatrienoic Acids (EETS) (Figure 1).* Epoxyeicosatrienoic acids (EETs) are arachidonic acid derived products of cytochrome P450 (CYP450) epoxygenases [33]. CYP450 enzymes are membrane-bound, heme-containing terminal oxidases in a multienzyme system. The arachidonic acid metabolizing CYP450 enzymes with prominent roles in vascular regulation are the epoxygenases of the CYP 2 gene family (e.g., CYP 2B, 2C8, 2C9, 2C10, and 2J2 in humans) that generate a series of region-specific and stereo-specific epoxides (5,6-, 8,9-, 11,12-, and 14,15-EETs), and the arachidonic acid v-hydroxylases belonging to the CYP 4A family which form hydroxyeicosatetraenoic acids (HETEs) which can demonstrate organ specific opposed actions [34].

Evidence in favor of EDHF being a short-lived metabolite of the cytochrome P450 epoxygenase pathway has been obtained using bovine [35, 36], porcine [36, 37], canine [38, 39], murine [40, 41], and human coronary arteries [8, 9, 29, 42–45]. EDHF-mediated responses are blocked by nonspecific CYP450 inhibitors such as miconazole, 17-octadecynoic acid, and more selective epoxygenase inhibitors [6-(2-proparglyloxyphenyl) hexanoic acid and *N*-methylsulphonyl-6-(2-propargyloxyphenyl) hexanamide] [46–48]. RT-PCR, Western blotting, and immunofluorescence techniques have demonstrated that native coronary endothelial cells express CYP epoxygenases, including CYP 2C8, CYP 2C9, and CYP 2J2 [49–52]. EET-induced activation of $K_{Ca}^+$ channels appears to be mediated by a cascade of intracellular events involving the ADP ribosylation of cellular proteins including anti-G(S)alpha antibody [53, 54] ultimately hyperpolarizing smooth muscle cells by increasing the open-state probability of $K_{Ca}^+$ channels [35, 41, 50, 55]. In porcine coronary arteries, a CYP450-derived epoxide, namely 11,12-epoxyeicosatrienoic acid (11,12-EET) has been shown to possess EDHF properties because (1) both 11,12-EET and bradykinin elicit hyperpolarization; (2) endothelial cells, but not smooth muscle cells, expressed mRNA and protein for the epoxygenase enzyme of the CYP2C family and released 11,12-EET; (3) induction of CYP2C8 or CYP2C34 increased epoxygenase expression, which was associated with increased release of 11, 12-EET, and enhanced relaxation and hyperpolarization in response to bradykinin; (4) an antisense oligonucleotide directed at the endothelial epoxygenase reduced both CYP2C mRNA and protein expression and the capacity to generate 11,12-EET, concomitantly with a reduction in the vasorelaxant and hyperpolarizing response to bradykinin [50]. The finding that sulfaphenazole, a selective inhibitor of CYP 2C9 [56, 57], inhibits EDHF-mediated responses [50] and potentiates non-NO-mediated relaxation in the porcine coronary artery [33] suggests that the CYP isoform required for the generation of EDHF is a porcine equivalent of CYP 2C9 [58]. Further, there is evidence in some species that CYP450-derived epoxides stimulate $Na^+/K^+$ ATPase [59]. These data strongly suggest that the activation of a cytochrome P450 epoxygenase is a prerequisite for the generation of EDHF-mediated relaxation in certain species.

Other intracellular second messenger roles of EETs may be equally as important in the control of vascular homeostasis. EETs (in particular 11,12- and 14,15-EET) activate several intracellular protein kinases including tyrosine kinases, the p38 MAP kinase, and extracellular-regulated protein kinases 1 and 2 (Erk1/2) and increase the proliferation of various cell types, including vascular smooth muscle cells and endothelial cells [33, 60, 61].

In isolated human coronary arterioles, CYP450-dependent hyperpolarization plays a significant role in the regulation of smooth muscle tone via activation of $K_{Ca}^+$ channels [8, 29]. Human coronary arteriolar endothelium-dependent hyperpolarization in response to arachidonic acid is much more dependent on metabolism by CYP450 than by

cyclooxygenase or lipoxygenase or activation of NO synthase [8, 62]. The predominant EET synthesized by arteries is 11,12-EET, and its specific inhibition by chemically distinct CYP450 inhibitors impairs EDHF relaxation. 11,12-EET activates large-conductance $K_{Ca}^{+}$ channel current and hyperpolarizes arterial smooth muscle. Large-conductance $K_{Ca}^{+}$ channels and CYP450-2C mRNA and proteins are less abundant in arteries than saphenous veins explaining the lack of EDHF activity of veins. Large-conductance $K_{Ca}^{+}$ channels are primarily present in vascular smooth muscle, whereas the CYP450-2C enzyme is present in both the endothelium and smooth muscle cells. Thus, in human internal mammary arteries, EDHF appears to be 11,12-EET, produced by an EDHF synthase CYP450-2C and accounts for 40% of net endothelial relaxation by stimulating large-conductance $K_{Ca}^{+}$ channels [6].

The role of EETs as potential EDHFs can be studied using azoles such as miconazole that selectively inhibit epoxidation (EET generation) of arachidonic acid and have been demonstrated to be partly responsible for endothelium-dependent vasodilation in the human microcirculation [9, 31, 63]. *In vivo* studies have demonstrated CYP450 inhibition does not alter conductance vessel diameter or resting blood flow [31, 63, 64], but after inhibition of NO and prostacyclin, inhibition of EET synthesis further decreases radial arterial blood flow and diameter [31]. Thus, although it appears that under resting conditions in the healthy human forearm, conductance and resistance vessel tone is not modulated by tonic activity of CYP450-derived epoxides, their role becomes evident after inhibition of NO and prostacyclin synthesis, illustrating the potential compensatory role of EETs on maintenance of basal tone when NO availability is diminished.

In recent studies, we have addressed previous controversies regarding the contribution of EDHF to resting vasodilator tone. In the largest cohort studied to date, an important contribution of EDHF, via activation of $K_{Ca}^{+}$ channels, to resting microvascular dilator tone in the human forearm *in vivo* has been demonstrated [65]. For the first time, we have also demonstrated that cytochrome P450-derived epoxyeicosatrienoic acids also contribute to resting vasodilator tone in the healthy microcirculation with the use of fluconazole to block their action. We found a relatively greater contribution of NO compared to EDHF in the maintenance of resting vasodilator tone in the healthy human forearm microvasculature. The contribution of these two endogenous vasodilators to resting tone differed in subjects exposed to risk factors for atherosclerosis, in whom the vasculature is characterized by decreased NO bioavailability. We found preserved contribution of EDHF that appears to contribute equally as much as NO to resting vasodilator tone in subjects with risk factors. In the presence of NO blockade with L-NMMA, epoxyeicosatrienoic acid-mediated microvascular vasodilation also appeared to be upregulated in healthy subjects [65]. This indicates potential cross-talk between the NO and EET pathways, such that EET activity is upregulated in the setting of NO deficiency which may be demonstrated by blocking NO synthesis in the healthy circulation. Interestingly, in the risk factor group, there was a similar contribution of EETs to resting tone in the presence and absence of NO blockade. Such compensation may be crucial in maintaining normal resting blood flow in nonhypertensive patients with risk factors.

Finally, by conducting experiments using single and combined blockade, we also demonstrated that $K_{Ca}^{+}$ channel activation contributes to microvascular dilator tone even after inhibition of epoxyeicosatrienoic acids. This indicates that sources other than epoxyeicosatrienoic acids contribute to hyperpolarization of the resting human forearm microcirculation. Potential candidates which have been investigated in experimental studies, include hydrogen peroxide, potassium ions, and gap junctions. These alternate pathways warrant further investigation *in vivo* in humans.

*2.3. Hydrogen Peroxide.* Hydrogen peroxide also activates calcium-dependent potassium channels and remains a contender as an EDHF [66] (Figure 1). Reactive oxygen species can increase $K^{+}$ channel activity and hyperpolarize smooth muscle [67, 68], and hydrogen peroxide may function as an EDHF [69]. Rubanyi and Vanhoutte reported that superoxide attenuates endothelium-dependent relaxations and that hydrogen peroxide causes endothelium-dependent and -independent relaxations [70]. Matoba et al. utilized catalase, an endogenous peroxidase to show inhibition of EDHF-mediated, endothelium-dependent relaxations and hyperpolarizations, resistant to indomethacin or N(omega)-nitro-l-arginine [71]. These findings have been confirmed in piglet pial arteries, canine subepicardial coronary arteries and arterioles, and during flow-induced vasodilation in human mesenteric arteries and coronary microvessels [66, 72–74].

Vascular endothelial cells have a capacity to produce superoxide and hydrogen peroxide from several intracellular sources, including endothelial NO synthase, cyclooxygenases, lipoxygenases, cytochrome P-450 epoxygenases, NAD(P)H oxidases, and xanthine oxidase [71, 75–78]. Flow-mediated dilation involves generation of superoxide originating from mitochondria and shear stress elicits luminal release of mitochondrial ubisemiquinone, a source for generating superoxide and hydrogen peroxide via metabolic processes occurring between complex I and complex III of the electron transport chain [79]. Although reactive oxygen species appear to fit the profile of EDHF, their physiologic role remains a subject of debate particularly in some human arteries because in human radial and internal mammary arteries, neither catalase nor superoxide dismutase inhibited relaxations to carbachol [80, 81].

Hydrogen peroxide also mediates hyperpolarization via activation of endothelial $K^{+}$ channels, however, many species variations exist in the type of $K^{+}$ channels that are activated [67, 68, 72, 73, 82–84]. Importantly, in human coronary microvessels, $K_{Ca}^{+}$ channels sensitive to charybdotoxin plus apamin appear to mediate hyperpolarization [73, 85]. In mouse mesenteric arteries, the inhibitory effect of catalase was unmasked by the inhibition of NO production, and vice versa, suggesting that NO and EDHF (hydrogen peroxide) compensate for each other [71, 86–89]. In canine subepicardial coronary arteries and arterioles, the response to

acetylcholine and hypoxia was inhibited by L-NMMA primarily in subepicardial coronary arteries, whereas combined infusion of L-NMMA plus catalase or tetraethylammonium attenuated the vasodilator responses of coronary arteries of both sizes, demonstrating the predominance of hydrogen peroxide-mediated hyperpolarization in microvessels [74].

*2.4. Gap Junctions.* The EDHF phenomenon may be further explained by the transmission of endothelial cell hyperpolarization to the vascular smooth muscle via gap junctions [90–92]. These are myoendothelial and heterocellular. They couple endothelial cells to other endothelial cells and to smooth muscle cells, providing a low-resistance electrical pathway between the cell layers. Gap junctions are formed by the docking of two connexons present in adjacent cells that creates an aqueous pore permitting the transfer of ions and electrical continuity that establishes a uniform membrane potential across cells [93, 94]. Their number increases with diminution in the size of the artery [95], paralleling the importance of EDHF to vessel size with a greater influence in the resistance than in the conductance vessels [96].

Investigation of gap junctions as other potential EDHF mechanisms has been limited in man due to the lack of specific pharmacological agents. Rotigaptide, that enhances communication via the connexin 43 gap junction subunit, had no effect on basal vascular tone endothelium-dependent (bradykinin), -independent vasodilation, or t-PA release in the forearm arterial circulation of healthy men [97].

*2.5. Potassium ($K^+$).* A moderate increase in the myoendothelial $K^+$ concentration can in some species [98] induce hyperpolarization of vascular smooth muscle cells by activating the inwardly rectifying $K^+$ channels and the $Na^+$/ $K^+$ ATPase [24, 99, 100]. However, it is unlikely that $K^+$ *per se* is EDHF.

## 3. Interactions between EDHF, Nitric Oxide, and Prostacyclin

The three main mediators of endothelial vasodilator function, NO, prostacyclin, and EDHF appear not to be mutually exclusive and act synergistically in a complex manner to maintain the health of the vasculature (Figure 1). In conduit arteries, NO is the predominant endothelium-derived vasodilator but has relatively less prominent contribution in the resistance vessels of the microcirculation where EDHF appears to predominate [96]. NO may tonically inhibit EDHF responses as some studies could only demonstrate EDHF responses once NO production had been inhibited [44].

## 4. EDHF and Disease

Experimental evidence indicates that a shift away from NO-mediated endothelium-dependent relaxation toward EDHF-dependent relaxation occurs in disease states [101, 102]. Alteration of EDHF-mediated responses has been reported with aging, hypertension, atherosclerosis, hypercholesterolemia, heart failure, angioplasty, eclampsia, diabetes, and sepsis. Depending on the vascular bed, this may either contribute to endothelial dysfunction or compensate for the loss of NO bioavailability [103–105]. In the human forearm of hypertensive subjects, Taddei and others demonstrated that endothelium-dependent vasodilation is maintained despite decreased NO bioavailability because of the compensatory increased activity of EDHF [25, 104, 105]. Hypercholesterolemia is generally associated with preserved EDHF responses where its enhanced activity may compensate for the decrease in NO-mediated relaxation [106–108]. En-dothelium-dependent hyperpolarization appears to be inhibited in isolated gastroepiploic arteries from atherosclerotic patients, an effect that may be secondary to the duration of hypercholesterolemic injury [4]. In contrast, EDHF-mediated responses are depressed in some models of type I and type II diabetes with the exception of murine models [109].

In the healthy forearm microcirculation, we demonstrated that bradykinin-stimulated vasodilation is in part mediated by activation of $K_{Ca}{}^+$ channels and that the magnitude of contribution of NO was less than the contribution of $K_{Ca}{}^+$ channel activation [65]. Importantly, we found no contribution of $K_{Ca}{}^+$ channel activation to acetylcholine-stimulated vasodilation in healthy subjects, either in the presence or absence of NO-blockade. Thus, acetylcholine predominantly stimulates the release of NO and no EDHF, whereas bradykinin stimulates release of both EDHF and NO. We also demonstrated a similar vcontribution of NO and EDHF to bradykinin-mediated vasodilation in both groups. In contrast to effects of bradykinin, forearm blood flow response to acetylcholine was diminished in hypercholesterolemic subjects when compared to healthy subjects. Moreover, in hypercholesterolemia, we observed a significant contribution of $K_{Ca}{}^+$ channel activation and a lower NO release with acetylcholine that was distinctly different compared to the healthy circulation. Thus, while in health NO is the predominant contributor, in hypercholesterolemia, both NO and $K_{Ca}{}^+$ channel activation contribute equally to acetylcholine-mediated microcirculatory vasodilation [65].

Evidence suggests that CYP expression [110–112] and EET generation are increased in hypertension [113, 114], during salt loading [115], and in hypercholesterolemia [116]. Members of the CYP 2C family are inhibited by NO, a phenomenon that may explain why EDHF-mediated responses are barely detectable in the absence of the combined inhibition of NO synthases and cyclooxygenase in normal vessels. Thus the EET/EDHF pathway may be of less importance in healthy vessels and of greater significance in disease states where NO activity is impaired [58]. A similar phenomenon has been described for bradykinin-induced changes in forearm blood flow in essential hypertensive patients [25] and in arterioles removed from patients with coronary artery disease, where vasodilatation is mediated entirely by a mechanism sensitive to both CYP and $K_{Ca}{}^+$ channel inhibitors [9]. Such findings indicate that in the absence of NO, vascular tone can be regulated by an EDHF-like mechanism. Thus, whether EDHF plays a causal or compensatory role in the endothelial dysfunction in the human circulation remains to be elucidated.

Table 1: Pharmacological inhibitors of EDHF. Pharmacological agents used as potential inhibitors of EDHF and their targets and limitations (modified from Torondel et al. [117]).

| Pharmacological inhibitors | Targets | Comments |
|---|---|---|
| Apamin | $SK_{Ca}^{+}$ | Highly specific |
| Charybdotoxin | $IK_{Ca}^{+}$-$BK_{Ca}^{+}$ | Can inhibit some Kv channels |
| Iberiotoxin | $BK_{Ca}^{+}$ | Highly specific |
| Tetraethylammonium | $SK_{Ca}^{+}$-$IK_{Ca}^{+}$-$BK_{Ca}^{+}$ | Inhibit other $K^+$ channels at $>10^{-2}$) m |
| Tetraethybutylammonium | $SK_{Ca}^{+}$-$IK_{Ca}^{+}$-$BK_{Ca}^{+}$ | Inhibit other $K^+$ channels at $>10^{-2}$ m |
| $BaCl_2$ | $K_{IR}^{+}$ | — |
| Ouabain | $Na^+/K^+$ ATPase | Can affect gap junction activity at $>10^{-4}$ m |
| KCL | $K^+$ currents | Dilates at $>10^{-2}$ m through $K_{IR}^{+}$ and $Na^+/K^+$ ATPase activation |
| 18 $\alpha$-glycyrrhetic acid | Gap junctions | Possesses nonjunctional effects on membrane currents |
| Connexin mimetic peptides | Gap junctions | Highly specific |
| Catalase | Hydrogen peroxide | — |
| 17-octadecenoic acid | CYP | Inhibits the synthesis of the vasoconstrictor 20-HETE |
| Clotrimazole | CYP | Can inhibit $K^+$ channels |
| Miconazole | CYP | Can inhibit $K^+$ channels |
| Sulphaphenazole | CYP epoxygenase | Highly specific of CYP 2C9 |
| Fluconazole | CYP epoxygenase | Can inhibit other CYP isoforms at $>10^{-4}$ m |
| MSPPOH | EETs synthesis inhibitor | Highly specific |
| 14,15-EEZE | EETs antagonist | Inhibits the vasodilator action of all EETs regioisomers |

$K^+$: potassium, $SK_{Ca}^{+}$: small calcium-dependent potassium channels, $IK_{Ca}^{+}$: intermediate calcium-dependent potassium channels, $BK_{Ca}^{+}$: large calcium-dependent potassium channels, Kv: voltage dependent potassium channels, $K_{IR}^{+}$: inwardly rectifying potassium channels, $BaCl_2$ barium chloride, KCL: potassium chloride, CYP: cytochrome, 20-HETE: 20-hydroxyeicosatetraenoic acids, MSPPOH: N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide, EETs: epoxyeicosatrienoic acids, and 14,15-EEZE: 14,15-Epoxyeicosa-5(Z)-enoic Acid.

## 5. Summary

Absence of consensus regarding the precise identity of EDHFs and a consequent lack of specific inhibitors has long hampered clinical translation of this phenomenon (Table 1). Recently, with improved understanding of the major signaling mechanisms underlying vascular hyperpolarization, the role of EDHF in the human circulation *in vivo* has begun to be dissected, but experimental pitfalls remain. These include the often nonspecific nature of the antagonists used, the concentrations and duration of action of these blockers are variable, and complete blockade cannot be achieved *in vivo,* even with high doses, because of the competitive nature of the antagonism. Nevertheless, an impressive body of knowledge has already emerged regarding the role of EDHF in the human circulation (Table 2).

Apart from its contribution to normal vascular physiology, the accentuated role of EDHF in diseased states is worthy of further investigation because CYP-450 expression and EET generation are increased in hypertension, during salt loading and in hypercholesterolemia. Vasodilation in essential hypertension [25], and in atherosclerotic coronary arterioles, is largely secondary to CYP and $K_{Ca}^{+}$ channel stimulation. There are also potential implications regarding disease susceptibility, with some polymorphisms within CYP epoxygenases being associated with an enhanced risk of developing coronary artery disease and hypertension [14]. What may ultimately be of even greater interest is development of specific agents targeting EDHF synthesis, understanding of other biological effects of EETs such as angiogenesis and modulation of cell growth, and their potential role in human disease [14].

## 6. Implications

Conventionally, endothelial dysfunction is characterized as a deficiency of NO activity, often secondary to exposure to cardiovascular risk factors. This leads to abnormalities in vasodilation and hence blood flow delivery. Because of the known protective role of NO on the vessel wall that impedes thrombosis and atherosclerosis, several strategies have been applied to improve NO availability/activity. Although replacing NO pharmacologically with NO donors is beneficial for symptomatic relief from coronary vasodilation, there appears to be no antiatherosclerotic effect of NO donors. Improving endothelial NO bioavailability with statins and angiotensin antagonists has nevertheless proven to be cardioprotective. What remains unknown is (a) whether enhancing EDHF in conditions with impaired NO activity would also be of therapeutic value, (b) whether agents that improve endothelial dysfunction (acetylcholine responses) such as statins and angiotensin antagonists, also enhance EDHF bioactivity, and (c) whether tissue plasminogen activator release is EDHF-dependent in health and disease. Indeed hypertension is associated with elevated epoxide hydrolase expression [128, 129], angiotensin II increases the expression

Table 2: Human vascular territories with characterized EDHF activity.

| Vascular territory | EDHF | Pharmacological agents used |
|---|---|---|
| *Preclinical studies* | | |
| Coronary arterioles [8, 9, 29, 73, 118] | $H_2O_2$, $K_{Ca}^+$ channels, CYP450 metabolites | Catalase, KCl, charybdotoxin + Apamin, polyethylene glycol catalase, KCl, charybdotoxin, 7-octadecynoic acid |
| Internal Mammary artery [6, 62] | 11,12-EET | 17-octadecynoic acid, N-methylsulfonyl-6-(2-propargyloxyphenyl)hexanamide4,15-epoxyeicosa-5(Z)-enoic acid |
| Gastroepiploic arteries [4] | $K_{Ca}^+$ channels | KCl |
| Mesenteric artery [66, 119] | $H_2O_2$, Gap junctions, superoxide dismutase, $H_2O_2$ | Catalase, 18 alpha-glycyrrhetinic acid, Tiron (cell-permeable SOD-mimetic), catalase |
| Renal artery [10] | $K^+$, $K_{Ca}^+$ channels | KCl, charybdotoxin, and apamin |
| Subcutaneous resistance arteries [7] | CYP450 metabolites, $K_{Ca}^+$ channels | Ketoconazole |
| Subcutaneous resistance arteries [120] (subcutaneous fat biopsies of healthy pregnant women) | Connexin 43 Gap junctions. | Connexin mimetic peptides |
| Visceral fat arterioles | $H_2O_2$ | Polyethylene glycol catalase |
| Umbilical vein endothelial cells [85, 121] | $SK_{Ca}^+$ channels, $IK_{Ca}^+$channels, $H_2O_2$ | Apamin and charybdotoxin/triarylmethane-34 |
| Thyroid arteries [122] | $K_{Ca}^+$ channels, $K_{IR}^+$ channels, $Na^+/K^+$ ATPase | Iberiotoxin, charybdotoxin, apamin glibenclamide, and barium |
| *Clinical studies* | | |
| Forearm microvasculature [63] | CYP450 metabolites | KCL, miconazole |
| Forearm microvasculature [30, 32, 123] | $K_{Ca}^+$ channels | TEA |
| Forearm microvasculature [124] | C-type natriuretic peptide | C-type natriuretic peptide, TEA |
| Forearm microvasculature [104] (hypertensive patients) | CYP450 2C9 | Sulfaphenazole |
| Forearm conductance vessel [31, 125, 126] | CYP 2C9 metabolites, $K_{Ca}^+$ channels | Sulfaphenazole, TEA, fluconazole |
| Thigh skeletal muscle vessels [127] | CYP450 2C9 | Sulfaphenazole |

$H_2O_2$: Hydrogen Peroxide, $K^+$: potassium, $SK_{Ca}^+$: small calcium-dependent potassium channels, $IK_{Ca}^+$: intermediate calcium-dependent potassium channels, $K_{IR}^+$: inwardly rectifying potassium channels, KCL: potassium chloride, CYP: cytochrome, and EETs: epoxyeicosatrienoic acids.

of the epoxide hydrolase [130], and epoxide hydrolase inhibitors are effective in reversing the hypertensive effects of angiotensin II [128]. Thus, epoxide hydrolase inhibitors that increase epoxide levels and hence aid hyperpolarization need to be further investigated in subjects with endothelial dysfunction.

Experimental studies indicate that cytochrome P450 expression and EET generation are increased in hypertension [104, 113], in hypercholesterolemia [116], and in atherosclerotic coronary arterioles [50, 64, 75]. Moreover, polymorphisms in the cytochrome P450 epoxygenase genes are associated with increased risk of coronary artery disease and hypertension [131, 132]. Thus, understanding the pathophysiology of endothelial dysfunction beyond NO, and in particular with respect to EDHF in these disease states, could be crucial in understanding both the pathophysiology of atherosclerosis and developing novel therapies.

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*Review Article*

# A Brief Journey into the History of the Arterial Pulse

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*Objective*. This paper illustrates the evolution of our knowledge of the arterial pulse from ancient times to the present. Several techniques for arterial pulse evaluation throughout history are discussed. *Methods*. Using databases including Worldcat, Pubmed, and Emory University Libraries' Catalogue, the significance of the arterial pulse is discussed in three historical eras of medicine: ancient, medieval, and modern. *Summary*. Techniques used over time to analyze arterial pulse and its characteristics have advanced from simple evaluation by touch to complex methodologies such as ultrasonography and plethysmography. Today's understanding of the various characteristics of the arterial pulse relies on our ancestors' observations and experiments. The pursuit of science continues to lead to major advancements in our knowledge of the arterial pulse and its application in diagnosis of atherosclerotic disease.

## 1. Introduction

> "Abu Ali placed his hand on the patient's pulse, and mentioned the names of the different districts and continued until he reached the name of a quarter at the mention of which, as he uttered it, the patient's pulse gave a strange flutter. Then Abu Ali repeated the names of different streets of that district and different houses till he reached the name of a house at the mention of which the patient's pulse gave the same flutter. Finally, he uttered the name of different households of that house until he reached a name at the mention of which that strange flutter resumed. Thereupon he said: This man is in love with such-and-such a girl, in such-and-such a house, in such-and-such a street, in such-and-such a quarter: the girl's face is the patient's cure" [1].

The above paragraph about Abu Ali Ibn Sina, known by his Latinized name Avicenna, a Persian scholar and a prominent physician of the Middle Ages, illustrating what was called "the quickened pulse of a lover" shows how physicians used the arterial pulse to diagnose certain illnesses in the medieval times. The simplicity of its evaluation had drawn even ancient physicians' attention to itself. Physicians of antiquity used the examination of the pulse not only for diagnosis, but also as an indicator of prognosis.

This paper will review the history of the assessment of pulse from ancient times to the present. The different methodologies for evaluation of arterial pulse characteristics will also be discussed briefly during three different historical eras of medicine, namely, ancient, medieval, and modern medicine.

## 2. Ancient Medicine

### *2.1. Indian Medicine.*

> "Immediately after pressing the pulse just below the hand joint, firstly, there is a perception of the beating of bayu; secondly, or between bayu and kaph, there is the perception of pitta; thirdly or the last, the perception of the beating of slesma or kaph is gained" [2].

This passage describes the examination and interpretation of the arterial pulse by ancient Indian physicians. Sage

Kanada (600 BCE), an ancient Indian physician, alchemist, and philosopher, in his book, "Science of Sphygmica", describes a variety of pulses during different physiological and pathological states. Based on his theory, each pulse has three stages, abnormality in any of which reflects diseases of three main humours of the human body, bayu/vata (air), pitta (bile), and kaph/kapha (phlegm) [3].

Ayurveda (knowledge of life) is an ancient medical science that has been originated in the Indian subcontinent and has been practiced since the time of Buddha (500 BCE). The examination of the pulse is an integral part of Ayurvedic medicine. Eight parts of a patient's body are described for physical examination, the first one being the arterial pulse [4]. A full description of the methodology of pulse examination has been described in three categories related to the examiner, the examined, and to the method of examination. A special method was introduced for counting pulse rate in Ayurvedic medicine. Heart rate was counted per "pal" with every 2.5 pal making a minute. Moreover, different pulse rates were described for different age groups in the Ayurveda [5].

*2.2. Chinese Medicine.* Arterial pulse was studied in China about two and a half thousand years ago. It was first mentioned in the "Internal Medicine Classics, Nei Ching". This manuscript is reported to be written by the Yellow Emperor, Huang Ti (698–598 BC). The principal means of diagnosis employed in the Nei Ching is the physical examination of the arterial pulse. The theory of the pulse is based upon the various stages of interaction between Yin (disease) and Yang (health).

The ancient Chinese physicians had to develop the ability and skill to judge the state of disease—its cause, duration, and prognosis—by the volume, strength, weakness, regularity, or interruption of the four main varieties of pulse beats (superficial, deep, slow, and quick) [6]. The examination was performed on both wrists, and the best time for examination was early in the morning, in that Yin and Yang were believed to be in balance [7]. The physician would judge the pulse rate based on the ratio between the beating pulse and respiration, four beats to one respiration being normal. The arterial pulse in ancient China was divided into three parts: inch (the one closest to the hand), cubit (the one further up in the arm), and bar (the one in between). Each of the pulse locations in each arm would represent the condition of two different organs of the body [8].

*2.3. Egyptian Medicine.*

> "There are canals (or vessels) in it (the heart) to every limb. Now if the priests of Sekhmet or any physician put his hands (or) his fingers on the head, on the hands, or his fingers on the back of the head, upon the two hands, upon the pulse, upon the two feet, he measures the heart, because its vessels are in the back of the head and in the pulse; because its pulsation is in every vessel of every member" [9].

The quotation above suggests that the relationship between the heart beat and the peripheral circulation was conceptualized in ancient Egyptian medicine [10]. It was thought that the arteries contain air, yet the whole concept of circulation was unknown [11]. It was also believed that there are two vascular systems in the human body: the veins, which carry the products of digestion from the digestive tract to the whole body and the arteries, which carry *pneuma* (air) from lungs to other organs. According to this system, the structure of the heart was inadequately explained. The left side of the heart was accounted for drawing the *pneuma* from the lungs and pumping it into the arteries of the rest of the body [12].

*2.4. Greek Medicine.* The word "artery" originated from the Greek word "$\alpha\rho\tau\eta\rho\acute{\iota}\alpha$" which seems to be derived from the word "$\alpha\eta\rho$" which means air [13]. The examination of the pulse was also described and defined in the Hippocratic writings. Despite Hippocrates (375 BC) being reported to describe the characteristics of the arterial pulse in several conditions such as fever and lethargy in his book on humors [14, 15], it was Praxagoras of Kos (340 BC) who was the first physician credited for examining the pulse in ancient Greek literature [16]. Praxagoras discovered that pulsation only occurs in the arteries, not in the veins [17]. His student, Herophilus (335–280 BC), was the first to describe pulsus caprizans, similar to the leap of a goat, an unusual type of pulse with two phases, an initial stroke followed by a stronger one. Another renowned physician of antiquity was Erasistratus (304–250 BC), a contemporary of Herophilus, who was mentioned by Galen as coming very close to the understanding of circulation [16]. Erasistratus stated that the heart and arteries do not move at the same instant, the arteries dilate while the heart contracts, and vice versa. He had recognized that the motion of arteries follows the contraction of the myocardium. Furthermore, Erasistratus explained correctly the dilation of the arteries as a passive expansion of the vessel yet incorrectly assumed that this was caused by the moving of *pneuma* along the course of the arteries. He and his contemporary physicians (Herophilus and Praxagoras) believed that arteries contain *pneuma*, while veins contain blood [18]. However, how the *pneuma* reaches the heart and arteries is not explained in any of the writings of this era [17]. Herophilus believed that dilation of the arteries draws in *pneuma* from the heart and contraction of the arteries moves it forward and this interplay generates the arterial pulse. In addition, the arterial pulse was thought to be inherent to the arteries and totally different from that of the heart [17]. This theory was believed until Galen, when arteries were discovered to contain blood in addition to air [19].

Herophilus was the first to compare the pulsation of blood vessels to musical rhythm and this theory had an enormous impact on both medical and musical literature until the late middle ages and the Renaissance. Upbeats and downbeats were the units Herophilus used to establish a basic analogy between musical rhythm and pulse rhythm. Herophilus defined the "perceptible time" as the interval of time in which the artery of a newborn would dilate. This

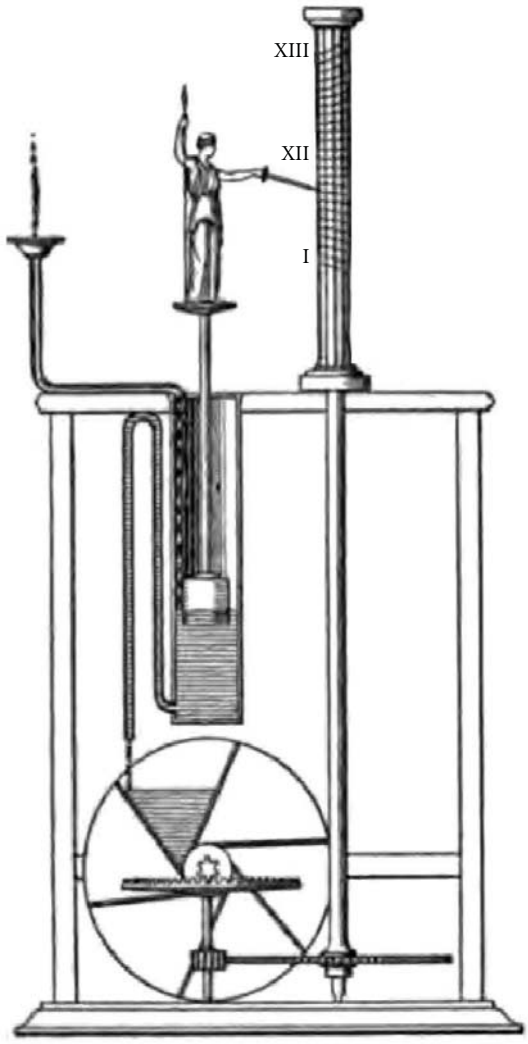



Figure 1: Clepsydra or Greek water clock: a portable water clock used by Herophilus for the purpose of arterial pulse examination. This water clock was capable of containing a specified amount of water for natural pulse beats of every age.

perceptible time became the basic unit by which the length of each contraction and dilation was measured, and hence the basic unit by which the pulse rhythm was established [17]. The most interesting part of Herophilus' clinical interest in the pulse is his unique construction of a portable water clock or *clepsydra* (Figure 1) which he used on his medical rounds to examine the pulse of his patients. This water clock would contain a specified amount of water for natural pulse beats of each age. Although Herophilus recognized the importance of determining the pulse rate, his followers failed to continue his studies in this respect, and there is rarely mention of pulse rate until the fifteenth century.

Archigenes (98–117 CE) discovered the dicrotic pulse as quoted by Horine [16]. It was thought by physicians of the Galenic era that the pulse has many variations, each variation carrying diagnostic or prognostic significance. Archigenes described each arterial pulse as having four characteristics (length, depth, breadth, and speed) and believed that these could be separated by careful palpation [17].

Galen (129–200 CE) thought that the dilation of the artery might be unequal on all sides and a variety of pulses could be felt based on the degree of dilation on each side. For instance, there could be a full upward dilation with a less lateral dilation making a high and narrow pulse. Likewise, there could be a full lateral dilation and a smaller one upward, resulting in a low and broad pulse. He described several types of arterial pulse such as saw-edged pulse, undulating pulse, and worm-like pulse. Other types of arterial pulsation in different temperatures or illnesses including hot pulses and cold pulses, the pulse of pain, inflammation, lethargy, convulsions, jaundice, and even of elephantiasis are described by him [16]. Unlike Erasistratus, Galen argued that the dilation and contraction of both the heart and the arteries are simultaneous. At the time of ventricular systole, the arteries are contracted too, and at the time of diastole they are expanded [20]. Galen described twenty-seven characteristics for a single beat of pulse based on its size, speed, and frequency. Galen's observation of the double-hammer pulse is interesting. He described it quite different from pulsus caprizans as having no interruption between two phases of dilation, the two phases being different in size with the second much smaller than the first [20]. To describe in any further detail all the different types of pulses distinguished by Galen is far beyond the limits of this paper. The principal work dealing with the pulse as such is his treatise in four books entitled "De Pulsuum Differentiis" [21].

## 3. Medieval Medicine

Arterial pulse continued to be one of the most important diagnostic and prognostic signs in medieval medicine. As an example, arterial pulse was mentioned to be valuable in prognostication of epilepsy. A medieval physician who felt the particular pulse of a patient suffering from epilepsy would project that the patient would have a seizure at some point during the natural course of the illness [22].

Avicenna (981–1037 CE) like his predecessors believed that health was based on the interplay of four different humours—blood, phlegm, yellow bile, and black bile [23]. In his treatise on pulse, Avicenna attributed the quality of pulse to the interaction of these four humours. Like Erasistratus, Avicenna thought that the contraction and dilation of both heart and arteries were simultaneous. Furthermore, he believed that arteries contain both blood and *pneuma* [23]. Following ancient Greek physicians, he also believed in four different movements in arterial pulsation: two movements and two pauses after each movement. However, unlike Galen he argued that the pause following each dilation to be difficult to perceive. He categorized the pulse based on its different characteristics and described different pulse modes in each of these categories:

(1) the size of dilation: strong, weak, and intermediate,

(2) the duration of each movement: short, long, and intermediate,

(3) the duration of the pause: hurried pulse, sluggish pulse, or intermediate pulse,

(4) the temperature of the pulse: hot, cold, or intermediate,

(5) the compressibility of the artery: easily compressible, incompressible, and moderately compressible,

(6) the fullness or emptiness of the artery: full of humour, containing no humour, and intermediate,

(7) the equality or inequality of force in consecutive beats,

(8) the regularity of the rhythm: regular or irregular (irregularly regular and irregularly irregular).

This classification is similar to what we know currently of the arterial pulse characteristics in arrhythmias such as atrial fibrillation. He described the irregularity both in a single pulsation and in a succession of pulse beats. In terms of irregularities of a single pulse beat, he described premature and dropped beats [24].

Avicenna came close to a general understanding of the various arrhythmias based on the characteristics of the pulse. He described different pulses similar to the pulses being observed in arterial and ventricular arrhythmias [25]. He categorized the arterial pulse more thoroughly than his ancestors. Moreover, he explained three main factors—vital power, resistance, and elasticity—to be of importance in making the size of a pulse beat. This was the first time that concepts like resistance and elasticity were defined in a physiological manner [26]. In describing different pulse rhythms, Avicenna also compared pulse rhythms to musical rhythms [27]. This belief that music is inherent in the beating of the pulse was widely believed throughout the Middle Ages [28]. He described several types of pulses in different age groups and in both genders. Likewise, he revoked different pulses during different physiological and pathological states. The mouse-tail pulse described by Avicenna is similar to what is known as "pulsus alternans" secondary to weakened myocardium. Undulating pulse, dicrotic pulse, and vermicular pulse are a few examples of different types of pulse that were described by him [29, 30]. Based on Avicenna's observations and descriptions of the arterial pulse, it is conceivable that he understood the pulse as a wave, rather than an impact generated by a cylindrical tube, a basic concept that is the foundation for the studies on the pulse wave in modern medicine.

"The pulse which is very abnormal and totally irregular demonstrates that the cause for its abnormal condition migrates".

This quotation from Moses Maimonides (1135–1204 CE) one of the most eminent physicians of the Middle Ages clearly shows that Maimonides understood various arrhythmias based on his findings of the pulse. Similar to his predecessors, he attributed these irregularities to the abnormal constitution of the humours of the heart. Furthermore, he correlated the arterial pulse rhythm with the severity of illness, with more severe illnesses having more irregular pulses [31]. He especially, described the arterial pulse of patients with ascites to be hard and small; however, that of patients with anasarca to be of a fluttering nature [31].

From the days of Hippocrates to the thirteenth century, physicians generally believed that the human heart consisted of four chambers. The lower chambers, the ventricles, were thought to contain blood and the upper chambers to contain air. It was believed that with pores between the two ventricles, venous blood coming from the liver would mix with the air coming from the lungs to make the vital *pneuma* flourishing the entire body through the arteries. It was Ibn al-Nafis (1213–1288 CE) who discovered the pulmonary circulation of blood. He contradicted the theory of invisible interventricular pores of Galen and others and introduced the pulmonary circulation to medicine [32]. Ibn al-Nafis in his commentary on the anatomy of the Canon, describes the blood to flow from the right chamber of the heart to the lungs via the pulmonary artery, then spreading through the lungs and becoming intermingled with air to create the vital spirit, flowing to the left chambers via the pulmonary veins and subsequently to the entire body [33–37].

## 4. Modern Medicine

"When I first tried animal experimentation for the purpose of discovering the motions and functions of the heart by actual inspection and not by other people's books, I found it so truly difficult that I almost believed with Francastorius, that the motion of the heart was to be understood by God alone. I could not really tell when systole or diastole took place, or when and where dilatation or constriction occurred, because of the quickness of the movement. In many animals this takes place in the twinkling of an eye, like a flash of lighting. Systole seemed at one time here, diastole there, and then all reversed, varied and confused. So I could reach no decision. Finally using greater care every day, with very frequent experimentation, observing a variety of animals, I felt my way out of this labyrinth, and gained information, which I desired, of the motions and functions of the heart and arteries…" [38].

From the 13th until the 16th century, when William Harvey (1578–1657 CE) discovered the greater circulation, there was no major advancement in the understanding of the physiology of the arterial pulse and circulation. Harvey fully described the circular blood flow in the body from the heart to the extremities via arteries and from extremities back to the heart via the venous system [39].

Although the arterial pulse had been an integral guide to reach a diagnosis in antiquity and medieval eras, general concepts of its generation were misunderstood. Both the heart and the arteries were thought to have their own pulsation and to contract simultaneously. It was thought before Harvey's dogma-shattering observations that the arterial pulse is the result of an active force generated in the arterial surface. It was William Harvey who for the first time attributed the generation of the arterial pulse to the contraction of the left ventricle and found the source of the heart beat in the right atrium. He contradicted his forefathers, Galen and Vesalius, in their belief of the origin of the arterial pulse in the arterial

wall and with his meticulous observations attributed the generation of the arterial pulse to a passive dilation caused by the blood inflow [40, 41] and compared this passive dilation of the arteries to the process of inflating a glove by blowing air into it [42, 43]. Furthermore, for the first time in the history of medicine, Harvey described the arteries and veins to contain nothing but blood [44, 45].

These major discoveries were made with the aid of his meticulous experiments on both humans and animals. In the "Anatomical Studies on the Motion of the Heart and Blood" he comprehensively described his experiments on a variety of animals such as snakes, frogs, snails, shell-fish, and fish [46]. A series of his examinations on the arterial pulse by applying a ligature to the upper extremity clearly shows that Harvey thought of the arterial pulse as a wave and not just an impact [47, 48]. This conceptualized idea founded on hypothesis-driven and methodological experimentation paved the path for studies of the pulse wave into modern times.

Quantitative hemodynamic measures like stroke volume, cardiac output, ejection fraction are described by him for the first time in the history of medicine [49]. It is evident that his insight into these physiological measures introduced more quantitative rather than qualitative observations into medicine, facilitating more revolutionary discoveries at a faster pace.

By taking a closer look at Harvey's description of the blood circulation, it will become obvious that nothing was known about the capillaries at that time except from an earlier description of the capillaries by Moses Maimonides as the narrow transits communicating arteries with veins. This observation came to the actual discovery of the capillaries by Marcello Malpighi, a professor of anatomy in the seventeenth century [50].

In regard to assessing the frequency of the pulse, the first physician who counted the pulse rate was Herophilus by using his *clepsydra*. After Herophilus and until the fifteenth century, there is no indication that the pulse rate was actually counted. It is reported that the writings of Bishop Nicholas of Cusa (1401–1464 CE) contains the earliest mention of counting the pulse though the method is not of the kind we use at present time [51]. Pulse rate was compared between a sick and a healthy individual based on the weight of the water flowing from a water clock's narrow aperture into its basin. One hundred pulse beats were counted in different individuals, and the weight of the water flowing to the basin was compared between them making the judgment of health and sickness possible [52]. Galileo's discovery of the pendulum in the early seventeenth century led to the invention of *pulsilogy* by Santorio Sanctorius (1561–1636 CE). This device consisted of a scale of inches and a cord with a movable weight marked with a transverse line (Figure 2). The physician would move the pendulum and note the pulse with his fingers. If the pendulum moved faster than the pulse, the physician would lengthen the line and vice versa until they would coincide, thus showing the pulse rate as the number of inches [51].

A century after the invention of the *pulsilogy*, John Floyer (1649–1734 CE) counted the pulse rate as we measure it today. He is credited as the first physician to use a pulse watch that would run for sixty seconds to count the pulse [53, 54]. After Floyer's introduction of the modern pulse count, physicians started to record their observations in their daily practice based on the number of pulse beats per minute.

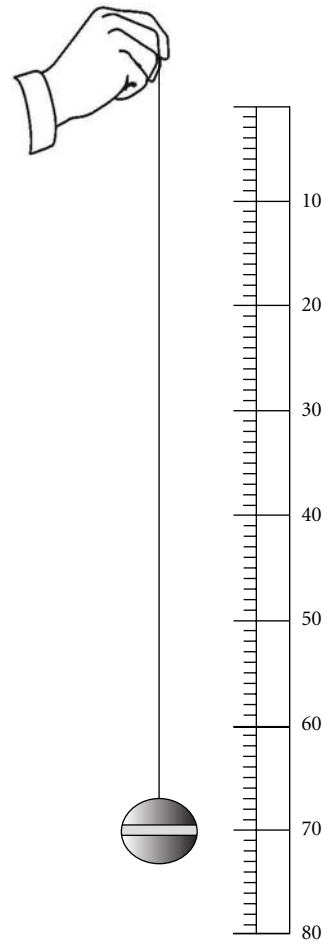



Figure 2: Pulsilogy of Sanctorius: this device consisted of a scale of inches and a cord with a movable weight marked with a transverse line. The physician would move the pendulum and note the pulse with his fingers simultaneously. Then, the physician would change the length of the line until the speed of the running pendulum would coincide with the pulse rate, thus showing the pulse rate as the number of inches.

Bryan Robinson, an Irish physician (1680–1754 CE), studied the pulse rate in different times during a day, and in people with different heights [55]. Jean Senac (1750–1770 CE), after studying one hundred soldiers, six feet in height, reported that the normal pulse rate ranges from 60 to 90 beats per minute [16]. Other observations were also made such as observations reporting the pulse rate to be a multiple of twelve [56], and those reporting the pulse rate to increase with age [57]. William Falconer (1744–1824 CE) in his great work called "Observations Respecting the Pulse" made numerous tables by which the degree of fever might be determined based on the proportion of the accelerated to the normal pulse [58]. The number of the pulse beats per minute

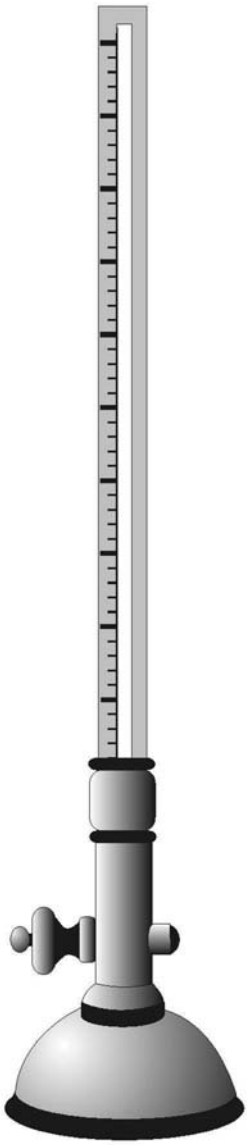


Figure 3: Herrison's sphygmometer: this device was composed of a graduate glass tube containing mercury with a semiglobular ball at one end. With the semiglobular end being placed over an artery, it would show the action of the vessel and the force of its impulse. It was designed in a manner that would enable the examination of the pulse in relation to its force, regularity, and rhythm.

and its characteristics on examination were the only methods of evaluating the pulse beat until the nineteenth century when Jules Herisson invented the *sphygmometer* (Figure 3). This instrument was composed of a graduated glass tube containing mercury with a semiglobular steel ball at one end. By placing the semiglobular end over an artery, the action of the vessel and the force of its impulse could be shown. This device was designed in a way that would make the examination of the pulse in terms of its force, regularity, and rhythm possible [59].

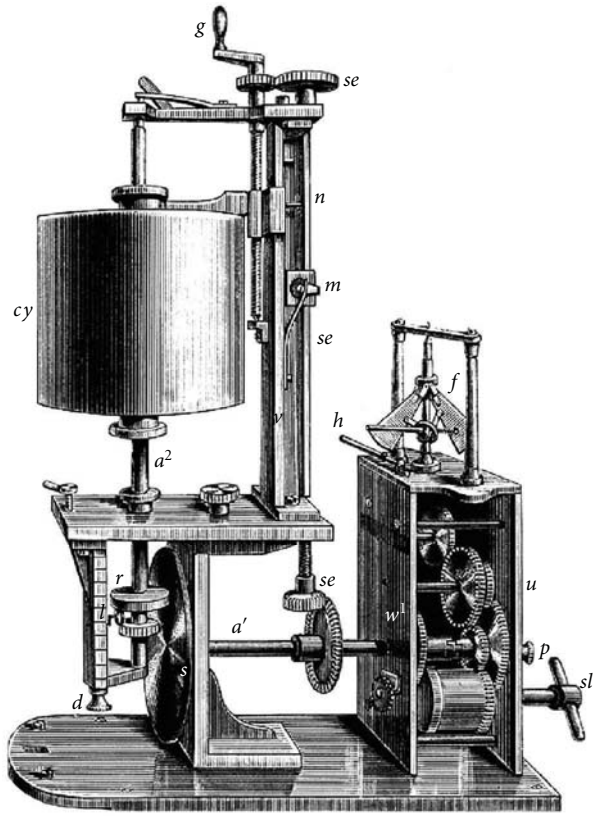



Figure 4: Ludwig's kymograph (wave writer): this device was able for the first time to graphically record hemodynamic measures.

The *sphygmometer* was the first device with the capability of showing the function of the heart and the arteries. Herisson in his book about his *sphygmometer*, for the first time argued that the sphygmometer was superior to the physicians' touch [60]. He described the sphygmometric findings in different valvular heart diseases like aortic, mitral, and pulmonary valve stenoses [61]. This was the first time that a physician would associate objective findings of a device with inner-body physiologic dynamics.

Twelve years later in 1847, Carl Ludwig, a German physiologist, invented the *Kymograph* which was a device capable of recording hemodynamic variables (Figure 4) [62]. Ludwig's introduction of the *Kymograph* in the middle of the nineteenth century greatly influenced the pace of cardiovascular research in future decades.

In 1860, the French physiologist Etienne Marey revised the previously invented *sphygmometer* and introduced his *sphygmograph* with graphical recording capabilities (Figure 5).

The first formal study on the arterial pulse wave was done by Marey with the help of his *sphygmograph*. He depicted the differences in arterial pulse waveforms in the elderly and younger adults [63]. Other examples on studies of the arterial pulse waveforms are Broadbent's lecture on the pulse and its characteristics during the examination [64], and an observation of the pulse rate, temperature, and weight with sphygmographic tracings performed on a man while he

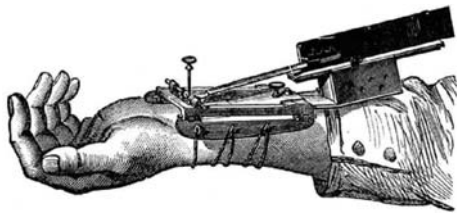


Figure 5: Marey's sphygmograph: this device had graphical recording capabilities of the arterial pulse. Applying this device to the wrist would record the motion of the arterial pulse and enable the examiner to interpret rate and rhythm.

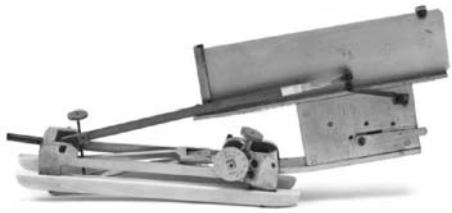


Figure 6: Mahomed's sphygmograph: this device was a revised form of Marey's sphygmograph with an added screw. It was capable of measuring the pressure needed to occlude the arterial wave along its graphical recordings of the arterial pulse wave dynamics.

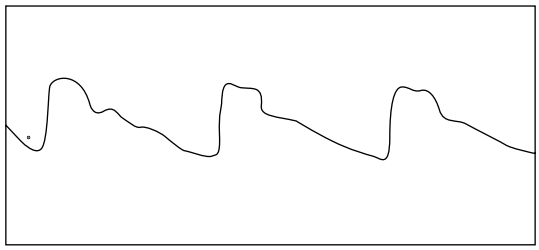
was walking a long distance by Mahomed (1849–1884 CE), Broadbent's student [65]. This served as a foundation for studies on the effect of ambulation on hemodynamic parameters such as pulse rate. Mahomed revised Marey's *sphygmograph* by adding a screw, thereby making the measurement of the pressure needed to occlude the arterial wave feasible (Figure 6). He used his revised *sphygmograph* to study the form and pressure of the pulse wave in several patients with different diseases [66, 67]. With the aid of his *sphygmograph* he described high tension in the arterial system and was the first to describe essential hypertension and distinguish it from hypertension caused by glomerulonephritis [68, 69]. In Figure 7, the graphical demonstration of the arterial pulse wave in asymptomatic patients with arterial hypertension is illustrated by O'Rourke [67]. Graphical recordings of the pulse in the nineteenth and early twentieth century permitted the arterial pulse to be studied as a wave and not just an impact [66]. Hawthorne, an early twentieth century physician made multiple observations based on his findings of the arterial pulse wave using both instruments. He argued that although at times the blood pressure findings may seem to be contradictory using both instruments, these two instruments were actually complementing each other [70]. Several forms of arterial pulse were described based on their sphygmographic characteristics in the twentieth century, that is, the water-hammer pulse, the bisferiens pulse, and the anacrotic pulse [71]. Mahomed's *sphygmograph* with the capability of quantitatively recording the pressure of the pulse wave led to the introduction of the sphygmomanometer in the late nineteenth century and the cuff sphygmomanometer by Postel-Vinay in 1896 [72]. With the advent of Riva Rocci's cuff sphygmomanometer, graphical tracing of the peripheral pulse wave gradually gave its place to peripheral blood pressure recording [73, 74]. Pulse wave tracing was difficult and time consuming with often times artefacts on recordings. On the other hand, the cuff would make the interpretation easier by just providing numbers that would reflect cardiac strength (systolic pressure) and arteriolar tone (diastolic pressure) [75]. A multitude of parallel studies and observations were done on the three major characteristics of the peripheral arterial pulse wave, its velocity, its pressure, and its volume. These led to the initiation of studies in clinical pharmacology on the application of certain medications on these physiologic parameters in the current era.

Modern studies on the pulse wave and arterial hemodynamics stem from the pioneering studies of the early modern era. Stephen Hales (1677–1746) in a series of papers called "Statical Essays: Containing Haemostatics" presented his contributions to the arterial hemodynamics before the Royal Society. The following quotation from his third experiment clearly shows the earliest studies on the mechanics of the circulation:

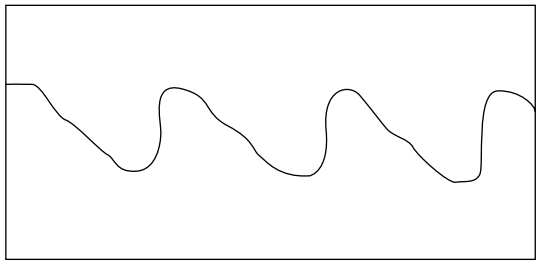
> "... But the systole of the ventricle during which that quantity of blood is propelled, being estimated to be done in one third of the space of time between each pulse, the velocity of the blood during each systole will be thrice as much, at the rate of 5211 feet, that is, 0.98 of a mile in an hour, or 86.85 feet in a minute" [76].

Hales's studies were followed by other scientists such as Leonhard Euler who formulated quantitative measures of the arterial hemodynamics. However, he was quite unsuccessful in proposing a formula that could simply show the relationship between the mechanical forces and the dynamic measures of the arterial pulse wave [77]. The idea of viewing the arterial pulse as a series of waves in a frequency domain had been related to the Fourier's heat wave transfer function studies. Similarly, the time domain analysis and the method of explaining the pulse mechanics with the characteristics of single pulse units had originated from the earlier works of Leonhard Euler (1707–1783). Euler's tube laws for arteries unsuccessfully tried to use a nonlinear form of conservation equations to explain the mechanics of the greater circulation. Years later, these were applied by Riemann to the time domain theory of the pulse wave.

Crighton Bramwell a scientist in the early twentieth century was first to introduce the concept of pulse wave velocity (PWV). He described the velocity to vary in proportion to the arterial wall tension and the blood pressure and to be an indirect measure of the arterial wall elasticity. Bramwell and Hill introduced a simple formula by which the arterial elasticity could be calculated from the PWV. With this formula he found a positive correlation between PWV and age and a negative correlation between arterial wall elasticity and PWV [78].



(a) Arterial pulse wave form in a man with arterial hypertension



(b) Arterial pulse wave form in a woman with arterial hypertension

Figure 7: Graphical depictions of the arterial pulse wave form by Mahomed in an asymptomatic male (a) and female (b) with arterial hypertension. Reproduced from [80].

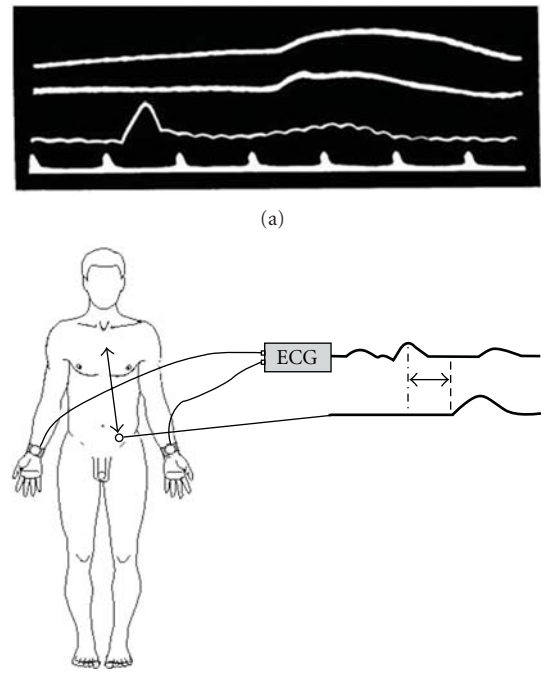
Bramwell's formula:

$$\text{Velocity (m/sec)} = 3.57/\sqrt{\%\ \text{increase in volume/mmHg increase in pressure.}} \quad (1)$$

During the mid-twentieth century, studies on hemodynamics of the arterial pulse wave were followed by the great works of Womersely and Nichols et al. [79, 80]. It is evident that with the advent of digital computers, Fourier's frequency domain analysis gained more popularity amongst scientists. Computers made calculations of the series of pulse wave transfer functions faster and easier. Taylor claimed that a more profitable approach had been made by using the frequency domain theory [81]. However, as time passed, again the time domain theory returned to studies on the dynamics of the arterial pulse wave. O'Rourke has fully described the application of the time domain analysis in clinical medicine and has compared it with the frequency domain. He has shown that modern analysis of the pulse in the time domain supports its historical perspectives and emphasizes the importance of arterial stiffening with age, wave reflection, their effects on the heart and their modification with therapy [82]. Despite all these studies, there is still an ongoing substantial controversy on the best method to be used in the clinical arena [83–85].

Since the invention of the *plethysmograph* by Schmitt in the mid-twentieth century [86], studies of the pulse wave have proceeded at a faster pace. Several methods have been in the past fifty years for the study of PWV, such as hot-wire sphygmography, impedance plethysmography, applanation tonometry, and recording the pulse wave using crystal microphones [87, 88]. While both sphygmography and plethysmography can be used for monitoring the pulse wave and its regularity, plethysmography can offer more technical usages including: peripheral oximetry, blood pressure monitoring and assessment of the adequacy of peripheral circulation as derived from the Allen's test [89]. In the 1960s Simmons and his colleagues applied a strain gauge plethysmograph to one digit and with the help of an amplified electrocardiogram recorded the resistance change caused by entrance of blood into that digit. Pulse waves from eighty normal subjects and patients with a variety of peripheral vascular diseases were recorded and compared in this study. They suggested that this technique could be of importance in the evaluation of peripheral arterial disease and the atherosclerotic changes of the peripheral circulation [90]. Crystal microphones were primarily used for determination of PWV. A crystal microphone would be applied to the groin over the femoral artery and would convert the pulse wave sound to an electric signal. Simultaneous EKG tracing would also be obtained and displayed. The time lag between the peak of the EKG's R wave and the foot point of the pulse wave would be used to calculate the PWV with inclusion of the distance between the two anatomical points of the patient's body in the equation (Figure 8) [91].

Woolam and his colleagues in 1962 used a crystal microphone for recording the pulse wave. By applying two crystal




(a)

(b)

Figure 8: (a) This graph illustrates time marker as the lowest line, cardiogram tracing as the line above that, and femoral pulse waves as the two top tracings. (b) Distance "a" represents the distance between aortic arch and the position of the microphone over the femoral artery "Reproduced from [91] with permission from BMJ Publishing Group Ltd."

microphones, one to the wrist and the other to the carotid artery they recorded the pulse wave and its velocity travelling along the course of the brachial artery. They demonstrated a higher PWV in diabetic patients. Other studies on the velocity of the arterial pulse wave showed that higher values are in a strong correlation with the atherosclerotic changes of the arteries [92, 93].

Gradually, studies of PWV changed from the peripheral circulation to the more reliable studies on the central circulation. Investigators started to study the pulse wave characteristics of the abdominal aorta mainly because it was more reliable, less variable, and more easily reproducible [93]. By using plethysmography for recording hemodynamics of the abdominal aorta, aortic PWV was found to be an important surrogate for stiffness of the coronary vasculature; this was confirmed with angiographic findings of the coronary vessels by Kogure et al. 1988 and Hirai et al. 1989 [94, 95]. In a recent meta-analysis of 17 longitudinal studies, Vlachopoulos and colleagues have found that aortic PSW is an independent predictor for all-cause mortality and cardiovascular outcomes and that risk is especially higher in patients with more baseline cardiac risks [96–99]. Recent studies suggest different effects on central blood pressure compared to peripheral blood pressure by some antihypertensive medications [100]. There remains still a considerable debate on which technology to use for prognostication. Future large-scale observational studies are needed to answer this specific question.

Asmar et al. in a study of 56 subjects compared a new automated device with manual calculation for determination of PWV and found that the findings from the two were very close and reproducible [101]. This finding led to the development of three automated devices: Complior (Artech Medical, Pantin, France), Sphygmocor (AtCor Medical, Sydney, Australia), and Arteriograph (TensioMed, Budapest, Hungary). Measuring PWV with Complior requires two pressure transducers that would record carotid and femoral pulse waves simultaneously. With calculation of the time delay between the two points, the machine will be able to provide an estimate for the velocity of the pulse wave. Sphygmocor utilizes a two step approach for calculation of the PWV with a single tonometer applied to femoral and carotid sites one at a time along with simultaneous EKG tracing. Arteriograph is a recently developed computerized device using oscillometric methods to determine PWV. The main principle used with Arteriograph is to record what is called the "return time". Return time is the time difference in milliseconds between the first wave and the reflected systolic pulse wave when cuff pressure is 35 mmHg over systolic blood pressure. Pulse wave velocity is then generated from the return time and the distance travelled by the wave. Arteriograph does not measure the time lag between carotid and femoral impulse. Rajzer and colleagues have compared the aortic PWV measured by these three devices in 64 patients with Stage I or II hypertension and have observed a difference in PWV derived from each method due to different estimation of the travelled distance of the pulse wave [102]. Jatoi and colleagues in a study of 254 untreated hypertensive patients compared PWV derived from Arteriograph with those of Complior and Sphygmocor and found that although the Arteriograph values were in close agreement with those of Complior and Sphygmocor, the techniques are not interchangeable [103]. Other noninvasive methods such as ultrasound and magnetic resonance imaging have been also introduced for the measurement of PWV [104–106]. Using ultrasound, aortic PWV was found to be an independent predictor of cardiovascular risk [107].

Doppler echocardiography with a high-resolution echo-tracking system is also used to estimate arterial compliance and augmentation index based on carotid artery diameter change [104, 108, 109]. The development of echo-tracking systems for estimation of arterial compliance was inspired by the work of Peterson and colleagues in 1960 who observed that vascular diameter pulse waveforms agree well with the intravascular pressure waveforms [110].

In contrast to applanation tonometry which can be applied to a limited number of peripheral arteries, the arterial distension waves obtained by echo-tracking systems can be recorded from a variety of sites and has an advantage of being very useful in obese patients for whom the applanation tonometry method might not be easily used.

Pulse pressure, another important characteristic of the pulse wave, has been studied extensively in more recent years. Its association with cardiovascular outcomes and its application in clinical pharmacologic studies are of clinical importance. As mentioned previously, with the invention of Riva Rocci's cuff sphygmomanometer in the early twentieth century clinicians made important observations regarding pulse pressure (pulsatile component of blood pressure) which was ultimately found to be an important risk factor for cardiovascular disease [111]. The positive association of pulse pressure with aging and arterial stiffness has also been scrutinized in several studies. The idea of this association was first brought about in the early nineteenth century by O'Rourke and Madhavan et al. who both diagnosed arterial stiffness by examining the characteristics of the arterial pulse waveforms [112, 113]. The Framingham study is one of the most important studies that has made seminal observations about the variation of blood and pulse pressure in different age groups. Furthermore, brachial pulse pressure was shown in different outcome studies to be a significant risk for developing coronary and carotid events [114–116].

Other than the pulse pressure, both the systolic and the diastolic components have been studied in relation to cardiovascular outcomes. Despite earlier misbelief that only systolic pressure is a predictor of outcome The Multi Risk Factor Intervention Trial (MRFIT) showed that both are independent predictors of cardiovascular outcomes [117].

Augmentation index (AI) as a measure of pulse pressure was first defined by Kelly and his colleagues in 1989. They defined AI for each pulse wave as the ratio of height of the peak above the shoulder of the pulse wave to the pulse pressure using a micromanometer probe over the carotid and radial arteries. They showed that the augmentation index was in direct correlation with age [118]. Several studies have shown an association between AI and increased cardiovascular risk [96, 119–121].

Table 1: Instruments used for the examination of the arterial pulse.

| Instrument | Inventor |
|---|---|
| Clepsydra | Herophilus (Third century BC) |
| Pulsilogy | Santorio Sanctorius (Sixteenth-Seventeenth century) |
| Pulse watch | Sir John Floyer (Seventeenth-Eighteenth century) |
| Sphygmometer | Jules Herisson (Nineteenth century) |
| Kymograph | Carl Ludwig (Nineteenth century) |
| Sphygmograph | Etienne Marey (Nineteeth century) |
| Hemotachometer | Karl Vierordt (Nineteenth century) |
| Plethysmograph | Otto Schmitt (Twentieth century) |

During the last twenty years, studies on the pathophysiology of atherosclerosis have shown endothelial dysfunction to play an integral role in the development of atherosclerosis in its preclinical stages. Endothelial dysfunction also has been shown to be an independent predictor of cardiovascular outcomes [122, 123]. Assessment of flow-mediated dilation of the brachial artery by ultrasonography, another characteristic of the arterial pulse wave and an indirect measure of pulse wave volume and arterial stiffness, has been introduced as a measure of endothelial function [124, 125]. Stiffness has been shown to be partly under control of the endothelium, which releases a number of vasoactive mediators. More recently, a reliable, less intensive, and technically simpler method of measuring endothelial dysfunction has been developed called pulsatile arterial tonometry (PAT). Pulsatile arterial tonometry is a novel technique to assess nitric oxide bioavailability and is less operator-dependent than flow-mediated dilation (FMD) for determination of endothelial function and arterial stiffness [126]. This device measures reactive hyperemia of the digits, which correlates with coronary microvascular endothelial function [127, 128]. The presence of coronary endothelial dysfunction is associated with lower PAT values in patients without obstructive coronary artery disease [127]. A linear relationship has been shown between PAT and brachial artery FMD measurements in patients with a variety of traditional risk factors [128].

Some of the most important instruments that have been used for the examination of the pulse throughout the history of medicine are listed with their inventors in Table 1.

## 5. Summary and Discussion

Current knowledge of the arterial pulse has culminated from the beliefs, observations, interpretations, dogmas, and the rejection of dogmas throughout the history of medicine. Our intention with this historical review is to make the reader appreciate how the understanding of the arterial pulse has progressed over the centuries to the present time and give the reader an insight for future developments.

In this paper, we describe the significance of the arterial pulse in clinical practice from ancient to modern eras. Several methodologies for the analysis of pulse wave and its characteristics including velocity, pressure, and volume have been discussed. These methods range from simple examination of the arterial pulse by touch to more complex techniques and devices that are being used and developed. Between all these methods, the only practical devices that have been shown in several studies to impact cardiac outcomes are cuff sphygmomanometer and noninvasive measurement of PWV. Despite its known limitations including limited precision, the cuff sphygmomanometer has held its place in routine evaluation and determination of arterial pulse pressure, globally. Despite the fact that measurement of PWV is recommended by the European Society of Cardiology as a prognostic factor [129], there is still lack of convincing clinical data to select and modify antihypertensive treatment based upon this clinical parameter [101, 130].

Scientific progress in arterial biology and physiology continues at a faster pace with the help of advanced technologies, designed by astute clinical observers, hypothesis-driven scientists and technical innovators; this body of work will certainly continue to grow and further our understanding of the arterial circulation.

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