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Endothelial Dysfunction in Human Essential Hypertension

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Published in:
Journal of Hypertension

DOI:
[10.1097/HJH.0000000000000965](https://doi.org/10.1097/HJH.0000000000000965)

Publication date:
2016

Document Version
Accepted author manuscript

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Mordi, I., Mordi, N., Delles, C., & Tzemos, N. (2016). Endothelial Dysfunction in Human Essential Hypertension. *Journal of Hypertension*, 34(8), 1464-1472. DOI: 10.1097/HJH.0000000000000965

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Journal of Hypertension

Endothelial Dysfunction in Human Essential Hypertension

--Manuscript Draft--

Manuscript Number:	JH-D-15-00477R4
Full Title:	Endothelial Dysfunction in Human Essential Hypertension
Article Type:	Review
Keywords:	endothelium; Hypertension; ACE inhibitors
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Abstract:	Although the endothelium has a number of important functions, the term endothelial dysfunction is commonly used to describe impairment in its vasodilatory capacity. It is increasingly recognised that this is related to hypertension, although whether it predates hypertension or is a consequence of it is still unknown. In this review we explore the mechanisms of endothelial dysfunction in hypertension, its prognostic significance and methods of pharmacological reversal.

NO	Nitric oxide
ACH	Acetylcholine
VEGF	Vascular Endothelial Growth Factor
eNOS	Endothelial nitric oxide synthase
ROS	Reactive oxygen species
FMD	Flow Mediated dilatation
ACE-I	Angiotensin converting enzyme inhibitors
ARB	Angiotensin receptor blockers
CCB	Calcium channel blockers

CONDENSED ABSTRACT

Endothelial dysfunction is prevalent in hypertension and has a recognised association with the condition. In this article we review the pathophysiology of endothelial dysfunction in human hypertension, its prognostic significance and methods of pharmacological reversal.



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08/04/2016

Professor Alberto Zanchetti
The Editor
Journal of Hypertension

Dear Professor Zanchetti

We thank you for the opportunity to resubmit our manuscript entitled “Endothelial Dysfunction in Human Essential Hypertension” (JH-D-15-00477R3) for consideration for publication in the Journal of Hypertension. We are resubmitting a revised version of the manuscript following the suggestions from the reviewer.

We appreciate the opportunity to revise this manuscript again and particularly would like to thank Reviewer whose constructive criticism has certainly improved the manuscript. We have addressed the minor comments suggested as attached to this letter.

We are extremely grateful once again to the reviewers and the editors for their detailed critique which has undoubtedly improved the quality of the manuscript.

We thank you once again for the opportunity to resubmit our manuscript and look forward to your decision.

Yours sincerely

Dr Ify Mordi

Reviewer #2:

I thank you the author for their last revision: the paper improved quite a lot! Just few points should be still addressed:

We thank the reviewer for their positive appraisal of our revised manuscript – we agree that the comments provided have substantially improved the review.

- Please change the sentence

“These features give the endothelium an important role in prevention of adverse cardiovascular outcome.”

to

“These features give the endothelium an important role in prevention of atherosclerosis development.”

We have changed this as suggested by the reviewer. (page 5, paragraph 1)

As the authors acknowledged in their review there is still no agreement about the possibility to prevent cardiovascular outcome by improving endothelial function.

- Please delete the following sentence or move it under the subtitle “invasive assessment of endothelial function”

„To study endothelial function, typically a number of substances can be given to the patient and the endothelial response assessed. Vasomotor function can be assessed by giving agonists which induce NO stimulation in the endothelium ("endothelium dependent" agonists such as acetylcholine) or endothelium-independent agonists such as sodium nitroprusside, which directly donate NO. Comparing these two aspects of endothelial function can provide important insights.”

We agree with the reviewer that this sentence is out of place. Given the length of the review and the fact that this is covered elsewhere we have now deleted the sentence.

- Please move the description of micromyography to the invasive methods as tissue biopsies are necessary.

We thank the reviewer for pointing out this error. We have now moved the description of myography to the invasive methods section (page 5).

Endothelial Dysfunction in Human Essential Hypertension

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Word Count 8,809 (including references)

We have no conflict of interest.

ABSTRACT

Although the endothelium has a number of important functions, the term endothelial dysfunction is commonly used to describe impairment in its vasodilatory capacity. It is increasingly recognised that this is related to hypertension, although whether it predates essential hypertension or is a consequence of it is still unknown. In this review we explore the mechanisms of endothelial dysfunction in essential hypertension, its prognostic significance and methods of pharmacological reversal.

Keywords: Endothelium; hypertension; ACE inhibitors

CONDENSED ABSTRACT

Endothelial dysfunction is prevalent in hypertension and has a recognised association with the condition. In this article we review the pathophysiology of endothelial dysfunction in human essential hypertension, its prognostic significance and methods of pharmacological reversal.

Essential hypertension is a worldwide epidemic, with approximately 20% of the world's population estimated to have a blood pressure greater than 140/90mmHg, and accounting for around 10% of worldwide healthcare costs^{1,2}. Due to its position as the leading risk factor for death worldwide, there is an increased drive towards its prevention³. Much of the adverse cardiovascular risk associated with hypertension is derived from its contribution to atherosclerosis, despite the two being distinct pathophysiological processes⁴.

Intuitively, the shared link between the two processes is the endothelium. Initially thought of as being a simple layer of cells in the vasculature separating blood from the interstitial space, it is now recognised as a key regulator of vascular health. Abnormalities in normal endothelial function are now recognised as a key part of both the atherosclerotic and hypertensive disease processes, sharing many common features.

Although the endothelium has a number of functions, the term “endothelial dysfunction” is usually used to refer to abnormalities in its vasodilatory capacity, and this is what we shall refer to in this paper. In this review we will discuss endothelial dysfunction, its links with hypertension and prognostic significance, before discussing treatment options and future direction.

What is Endothelial Dysfunction?

The endothelium responds to a number of endocrine, paracrine and autocrine signals to regulate these functions. The first evidence of this was described by Furchgott and Zawadzki who identified that the large blood vessels of rabbits only relaxed in response to acetylcholine if the endothelium was intact due to what they called endothelium-derived relaxing factor – which we now know as nitric oxide (NO)⁵. In fact, the endothelium releases a number of vasodilating and vasoconstricting factors with a local paracrine action. However, the healthy endothelium has a naturally vasodilated resting state, mainly due to the action of NO. NO is produced by the endothelium from L-arginine via the enzyme nitric oxide synthase in

response to numerous factors including mechanical shear stress. NO passes into the underlying smooth muscle by diffusion, stimulating guanylate cyclase to cause increased cyclic GMP production and thus causing vasodilation⁶. NO has a number of vascular protective functions, impairment of which could have damaging cardiovascular consequences. It is a strong inhibitor of platelet aggregation and adhesion, giving it anti-atherosclerotic properties. It also inhibits adhesion to leukocytes to the vessel wall and proliferation of vascular smooth muscle cells⁷. These features give the endothelium an important role in prevention of atherosclerosis development.

Any damage to the endothelium can cause impairment of its normal function i.e. endothelial dysfunction. This process is characterised by an imbalance in endothelium-dependent vasodilation and vasoconstriction and can occur by one of 3 methods.

1. Reduced production of NO; for example caused by reduced endothelial nitric oxide synthase (eNOS) activity due to asymmetric dimethylarginine (ADMA), a competitive inhibitor of eNOS⁸.
2. Reduced availability of NO; caused by reactive oxygen species (ROS) which convert NO to peroxynitrite which does not cause vasodilatation.⁹
3. Antagonism of NO by endothelium derived contracting factors.¹⁰

Assessment of Endothelial Function

Clinically endothelial function can be measured both invasively and non-invasively, and these have recently been reviewed in detail by Flammer et al.¹¹ We will briefly describe some of these techniques.

Invasive Assessment of Endothelial Function

Invasive measurement of endothelial function can be conducted in the coronary arteries by quantitative angiography. This can be supplemented by intravascular ultrasound for more detailed assessment of the vasculature and intracoronary flow.^{12, 13}

Venous occlusion plethysmography is another commonly used technique for assessment of endothelial function. The technique can theoretically be performed in any vascular bed, but is most commonly performed in the forearm circulation, by cannulation of the brachial artery.¹⁴ Venous return in the forearm is prevented by inflation of a cuff to above venous pressure but below diastolic arterial pressure (e.g. 40mmHg) with the forearm placed above the heart. The cuff is then deflated. A further cuff is inflated at the wrist to exclude the effects of the hand circulation. Changes in blood flow in the forearm can be measured using a plethysmograph. The concept relies on the concept that if (venous) blood flow out of the forearm is restricted but (arterial) inflow is maintained, the forearm swells at a rate in proportion to blood flow. As well as using plethysmography, ultrasound can also be used to measure arterial blood flow velocity and diameter (and hence flow).

Endothelial function can also be measured in the microvasculature. This is usually conducted by obtaining subcutaneous tissue biopsies, dissecting out small vessels and measuring function using micromyography (therefore it is an ex-vivo method to measure endothelial function).¹⁵ There have been varying results in studies correlating microvascular endothelial dysfunction and larger artery dysfunction, although this might be expected in different vascular beds.¹⁶⁻¹⁸

Non-Invasive Assessment of Endothelial Function

The commonest non-invasive technique for evaluation of endothelial function currently in use involves brachial ultrasound during reactive hyperemia assessing flow mediated dilation (FMD). This involves interruption of blood flow in a peripheral artery (most commonly

brachial) using a cuff to cause temporary ischemia. Release of the cuff causes an increase in blood flow and shear stress, leading to NO release and vasodilatation. The change in diameter of the blood vessel can be measured by ultrasound and is directly related to NO bioavailability, this providing a measure of endothelial function.¹⁹ This technique is particularly attractive as it is easy to repeat in patients, however there is still some variation between centres regarding image acquisition, for example in the time when measurements are obtained after cuff release so there still needs to be some standardization of the technique, though there have been attempts to do that.^{20, 21} Recently, FMD has shown good, reproducible results in multi-centre studies when rigorous methodology is used, perhaps suggesting that it will soon be able to be utilised in large prospective population studies as an outcome measure.^{22, 23} In a seven-centre study by Ghiadoni et al, the authors evaluated 135 adult healthy volunteers to assess reproducibility of FMD using a standardized protocol at 3 timepoints (baseline, 1 hour after baseline and 30 days after). They found that the technique was extremely reproducible across all sites, indicating that FMD could be used as a valid trial endpoint.²² These findings were extended in a further multi-centre study by Charakida et al who also evaluated patients up to 9 months after baseline FMD evaluation, and again the technique showed excellent reproducibility.²³

Peripheral artery tonometry (PAT) is a technique that non-invasive assessment of vasomotor function by plethysmographically measuring changes in the fingertip pulse (most commonly) as a surrogate for arterial tone. Again, the endothelium-dependent response can be ascertained by reactive hyperaemia following arterial cuff occlusion.²⁴

The cold pressor test was initially described in the 1930s by Hines and Brown.²⁵ To summarise, the test involves the immersion of the patient's hand into a bowl of ice-cold water, which causes increased sympathetic activity, leading to peripheral vasodilatation and a rise in blood pressure. Additionally, this also causes coronary vasodilatation and an increase in myocardial blood flow in healthy patients.²⁶ Therefore, using this technique, vascular

endothelial function can be assessed in a number of ways. Non-invasive methods include measures of blood pressure, peripheral arterial diameter,²⁷ echocardiographic coronary artery diameter²⁸ and techniques to evaluate myocardial blood flow such as PET²⁶. Additionally, invasive techniques can be used to assess changes in coronary artery size during coronary artery catheterisation.²⁹

More recently, laser Doppler has been utilised to measure endothelial function. This technique allows for non-invasive measurement of the microvasculature of the skin by assessing skin blood flow.³⁰⁻³² This technique uses the backscatter provided by moving erythrocytes when they reflect a laser signal scanned across the skin. The erythrocytes reflect the signal back at a frequency proportional to their velocity hence blood flow can be measured.

What is the Evidence for Endothelial Dysfunction in Hypertension – Cause or Effect?

Demonstration of impaired endothelial dysfunction in hypertensive patients in the forearm resistance vessels was first reported, almost simultaneously in the early 90s, by Panza et al³³ and Linder et al.³⁴ In the study by Panza et al, the authors studied changes in forearm blood flow in 18 hypertensive patients and 18 normotensive control subjects in response to acetylcholine and found that this response was impaired in the hypertensive patients. Linder et al evaluated 14 stable outpatients with essential hypertension (compared to 20 controls) and also found that the response to acetylcholine was blunted in hypertensives compared to controls. These findings have since been confirmed in patients with both essential and secondary hypertension.³⁵⁻³⁸

For a long time it has been thought that hypertension caused endothelial dysfunction. Since the first study by Panza et al³³ there have been numerous attempts to identify the mechanisms linking hypertension and endothelial dysfunction, however they are still not yet completely understood. There is a significant genetic component to the development of hypertension, and

Taddei et al used this to try and identify a common pathway in the adult offspring of hypertensive patients³⁹. In this study the authors found that the normotensive offspring of hypertensive patients had significantly impaired response to ACh in comparison to normotensive offspring of normotensive patients due to a defect in the L-arginine-NO pathway. Further evidence of a link between endothelial dysfunction and hypertension was obtained from the Framingham data⁴⁰. In this study systolic blood pressure was significantly inversely correlated with FMD, although the design of the study did not allow for determination of causation. Another study found that the presence of elevated blood pressure in a cohort of Finnish teenagers was predictive of impaired FMD after 21 years of follow-up⁴¹. Transient experimentally-induced hypertension has been shown to cause acute impairment of endothelial function in normotensive individuals⁴².

The mechanism of by which hypertension might cause endothelial dysfunction has been evaluated in various studies which have proposed a number of pathways, although many are in animals and of course caution is advised in extrapolation to human studies. Increased blood pressure causes an increase in superoxide production and a decrease in NO bioavailability^{9,43}. A second mechanism involves the renin-angiotensin system. Angiotensin-converting enzyme (ACE) acts on the endothelium converting angiotensin I to angiotensin II. This causes vasoconstriction and also promotes production of endothelin, another potent vasoconstrictor. An increase in both of these vasoactive substances manifests as endothelial dysfunction⁴⁴. However, the adverse prognostic effects of angiotensin II are not purely due to vasoconstriction. Indeed, hypertensive patients tend to have similar levels of plasma angiotensin to normotensives⁴⁵. In fact, angiotensin II also stimulates production of superoxide which leads to peroxynitrite formation. Peroxynitrite oxidizes arachidonic acid to form isoprostane, a potent vasoconstrictor.

The hypothesis that hypertension is a consequence of endothelial dysfunction has also been explored. A dysfunctional endothelium with poor vasomotor function would be in a state of

predominant vasoconstriction leading to a higher resting blood pressure. In one of the largest studies to date, Rossi et al examined 952 post-menopausal normotensive women and found that the risk of development of hypertension over the 3.6-year follow-up period was significantly higher in patients with low FMD compared with those with the highest at baseline.⁴⁶ This was however a study in a low-risk population in women and it is not clear if these results can be extrapolated to higher risk populations and males. Another recent study by Weil et al suggested that individuals with “pre-hypertension” (defined as BP 120-139/80-89) also had impaired endothelium-dependent vasodilation, which the authors speculated might lead to the development of hypertension.⁴⁷

It is likely that the relationship between endothelial dysfunction and hypertension is not sequential, but rather a cyclical one. Worsening in one may lead to worsening in the other, in a “vicious cycle” effect. Therefore, it could be important to establish its prognostic significance, if any, and consider treatment options.

The Prognostic Significance of Endothelial Dysfunction in Hypertension

Endothelial dysfunction has been shown to be an adverse prognostic indicator in several studies, for example in patients with coronary artery disease^{48, 49}, heart failure^{50, 51} and peripheral vascular disease⁵². Fewer studies have been conducted specifically in hypertension however. One of the earliest studies investigating the prognostic value of endothelial dysfunction in hypertension was carried out by Perticone et al.⁵³ In this study the authors examined 225 patients with untreated hypertension and found that the only independent predictors of future cardiovascular events were mean 24-hour blood pressure and endothelial dysfunction measured by forearm blood flow response using FMD. Hypertensive patients who have shown an improvement in endothelial function following intensive antihypertensive treatment also appear to have a better outcome than those in whom endothelial function does not improve.⁵⁴ Despite these promising early studies there have been no other large trials replicating these results. Some doubts about the generalizability of the study by Perticone et

al. could be raised, particularly regarding the surprisingly high event rate for what would be expected to be a low-risk population (4.9%/year). Indeed, conflicting results to these have been found in one smaller study.⁵⁵ All in all, it has not yet been established that endothelial dysfunction can be used as a predictor of adverse cardiovascular outcome in hypertensive patients, despite the evidence for its existence.

Pharmacological Reversal of Endothelial Dysfunction in Hypertension

Given the potential prognostic significance of endothelial dysfunction in hypertension, many studies have been conducted to improve endothelial function in this group of patients. The obvious starting point is with anti-hypertensive therapy. All of the mainstays of antihypertensive therapy have been extensively studied.

The Renin-Angiotensin-Aldosterone System

The majority of work has been conducted in drugs affecting the renin-angiotensin-aldosterone system. Angiotensin II stimulates angiotensin type 1 receptors (AT₁) to mediate arteriolar vasoconstriction and remodelling, superoxide anion production, renal sodium reabsorption, aldosterone secretion and endothelin (ET-1) release.⁵⁶ Many of these actions affect the vascular endothelium adversely. On the other hand stimulation of the angiotensin type 2 (AT₂) receptor by angiotensin has mainly opposing actions to those of AT₁ stimulation and recently has been shown to contribute to endothelial NO release.⁵⁷ Hence, AT₁ receptor blockade could improve NO bioactivity.

Angiotensin II can be reduced by angiotensin converting enzyme inhibitors which also increase both tissue and plasma bradykinin by inhibiting kininase II.⁵⁸ By stimulating the B2 receptors, bradykinin mediates the release of NO, prostacyclin and the endothelial hyperpolarizing factor (EDHF); agents that produce vasodilation.⁵⁹⁻⁶¹ ACE-Is (angiotensin converting enzyme inhibitors) and ARBs (angiotensin receptor blockers) are among the first-line therapies for hypertension.⁶² Two large studies have investigated the effects of ACE-Is

and ARBs in the coronary and forearm vasculature (TREND, BANFF) respectively in patients with atherosclerosis, showing improvement in endothelial function.^{63, 64} Several studies have confirmed this beneficial effect in hypertensive patients. Two independent long-term studies evaluating respectively the vascular responses to cilazapril and lisinopril on subcutaneous arteries (small resistance vessels) in essential hypertensives showed a considerable improvement in stimulated NO release following prolonged treatment with these agents.^{65, 66} Similar results were obtained in the coronary microcirculation of hypertensive patients with ACE-I. Antony et al showed that intravenous perindoprilat (the active metabolite of perindopril) restored the cold pressor test response and flow-mediated coronary artery vasodilation in 10 patients with untreated (newly diagnosed) essential hypertension.⁶⁷ Taddei et al have reported that 1 year of treatment with lisinopril improved endothelium-dependent and independent vasodilatation in forearm vasculature of hypertensives.⁶⁸ Several other studies also report improvement in endothelial function following ACE inhibition.⁶⁹⁻⁷³ However, there have also been studies showing conflicting results.^{74, 75}

There are also reports of the beneficial effects of ARBs on endothelial function. Schiffrin et al reported that one year of treatment with losartan improved both functional and structural properties of small resistance (subcutaneous) arteries in hypertensive patients.⁷⁶ Ghiadoni et al found that 1 year of treatment with candesartan improved stimulated NO release, but this was accompanied by a concomitant endothelium-independent improvement suggesting an alteration in vascular smooth muscle cell responsiveness.⁷⁷ Tzemos et al also found that valsartan mediated an improvement in endothelial function through both NO-dependent and independent pathways.⁷⁸ This is slightly contrary to a study by Klingbeil et al who found that while valsartan causes an increase in nitric oxide production in hypertensives there was no improvement in ACh mediated vasodilatation.⁷⁹ The authors postulated that the changes in ACh mediated vasodilatation may take longer to occur in hypertension and therefore require a longer period of treatment (the patients in this study were treated for 6 weeks as opposed to

16 weeks in the study by Tzemos et al.). Further studies have also shown beneficial effects on endothelial function with ARBs.⁸⁰

In summary, blocking the renin-angiotensin-aldosterone system either at the conversion from angiotensin I to II or at the angiotensin II receptor level may favourably affect NO bioactivity. It is likely that in the forearm microcirculation the tissue renin-angiotensin system is the predominant generator of angiotensin II hence only ACE-Is with high tissue penetration (enough to block the tissue renin-angiotensin system) are likely to be effective^{63,81}. In a sub-analysis of the recent EUROPA study (examining the beneficial effects of ACE-inhibition in patients with stable coronary artery disease), perindopril appeared to increase expression and activity of eNOS by 19% and 27% respectively, as well as a significant reduction in plasma levels of angiotensin II, tumour necrosis factor- α and an increase in bradykinin, nitrate and nitrite⁸².

In summary, it would appear that ACEIs and ARBs overall have beneficial effects on endothelial function.⁸³⁻⁸⁷

Spironolactone and eplerenone, mineralocorticoid receptor antagonists, have received much attention recently. They are recommended as 3rd or 4th line therapy in hypertension.⁶² They have been reported to improve NO bioactivity in patients with heart failure.⁸⁸ The mechanism(s) by which aldosterone impairs endothelial function is unclear. Aldosterone it is known to enhance vascular responsiveness to pressor agents such as norepinephrine and angiotensin II even before the systemic blood pressure begins to increase.⁸⁹ In patients with secondary hypertension due to aldosterone producing adrenal adenomas, surgical excision of these tumours removed the excess circulating aldosterone resulting in a normalised acetylcholine vascular response, i.e. improved endothelial function and BP control.⁹⁰

While there have been several animal studies showing beneficial effects of aldosterone blockade⁹¹⁻⁹⁴, there have been fewer in humans and results have been mixed. Savoia et al⁹⁵ conducted a randomised controlled trial comparing eplerenone to atenolol in 16 patients for 1 year and concluded that eplerenone treatment was associated with reduced arterial stiffness, decreased collagen/elastin ratio, and a reduction in circulating inflammatory mediators. While the study by Savoia et al and one by Yamanari et al⁹⁶ did not show that this improvement in endothelial function with aldosterone antagonists translated to humans, a more recent study by Fujimura did find an improvement in FMD with eplerenone⁹⁷. A further study in 320 post-menopausal women with hypertension by Rossi et al reported that use of aldosterone receptor antagonists (in conjunction with routine anti-hypertensive therapy) was the only drug class independently associated with improvement in FMD after 6 months.⁹⁸

Aliskiren, a novel direct renin inhibitor, has been recently studied in humans by Viridis in a comparative study against ramipril.⁹⁹ Aliskiren produced a significantly greater reduction in augmentation index and improved the vasodilatory response to acetylcholine, however blood pressure and pulse wave velocity reduction was the same in both groups. Dorresteijn et al also suggest that aliskiren may provide benefits in endothelial function in comparison to moxonidine or hydrochlorothiazide.¹⁰⁰ These results may provide interesting directions for further research, although aliskiren cannot as yet be recommended as anti-hypertensive therapy.

Dihydropyridine Calcium Channel Blockers

These are also recommended as first-line antihypertensive therapies.⁶² Calcium channel blockers (CCBs) have been shown to have a beneficial effect on nitric oxide bioactivity in coronary, forearm and resistance (subcutaneous) vascular beds.⁷¹ Perticone et al showed that oral isradipine also improved both stimulated (ACh-induced) and basal NO release in hypertension.¹⁰¹ Taddei et al demonstrated contrasting effects on the endothelium with acute and chronic administration of a highly lipophilic CCB, lacidipine.¹⁰² This group showed that

intra-arterial lacidipine did not significantly affect acute NO bioactivity, but chronic (2 and 8 months) oral treatment with this agent significantly increased vasodilatation in response to acetylcholine and bradykinin compared to baseline. Interestingly, this improvement in endothelial function persisted 2 weeks after drug withdrawal, despite the patients returning to hypertensive state. This suggests that improvement in endothelial function may be separate to blood pressure control, or that the effects of medications on each may have different durations. Similar improvements in endothelial function have been seen in other studies also.^{103, 104}

The mechanism by which CCBs improve endothelial dysfunction is unclear, although several mechanisms have been postulated. CCBs act directly on voltage dependent L-type calcium channels found on the vascular smooth muscle cells. Blockade of these channels reduces the influx of extracellular calcium into the sarcoplasmic reticulum facilitating vascular smooth muscle cell relaxation. However, endothelial cells are devoid of these receptors for calcium channel blockers to act on. In the coronary microcirculation the addition of a calcium channel blocker appears to indirectly facilitate an increase in intracellular smooth muscle cell cGMP, which is the second messenger of NO and mediates its vasodilation. Therefore, as expected an increase in intracellular cGMP with calcium channel blockers enhances both endothelium dependent and independent vasodilation.^{105, 106} This has been confirmed with both dihydropyridine and non-dihydropyridine calcium channel blockers. In contrast, in the forearm microcirculation, calcium channel blockers do not appear to influence the NO-independent pathway whenever exogenous organic sources of NO such as nitrates¹⁰⁷ are present.

Two additional mechanisms have been described to explain the effects of calcium channel blockers in the forearm circulation. The first explanation is that most calcium channel blockers have antioxidant activities, reducing production of superoxide anions.^{88, 89} The second explanation involves a reduction in endothelin-1 release by calcium channel blockers.

Normally, there is a balance between vasoconstrictive and vasodilating substances in the vasculature but in hypertension, the bioavailability of endothelin might be increased in parallel with a reduction in NO bioactivity. Cardillo et al have recently shown that in patients with essential hypertension, the increased endothelin activity is partly responsible for the increased vascular tone.¹⁰⁸ It has been shown that calcium channel blockers improve NO bioactivity by reducing endothelin release.^{100, 101} Hence, in a model where vasoconstrictive activity is increased, such as hypertension, a reduction of endothelin release would improve NO bioactivity. CCBs may also improve other aspects of endothelial dysfunction, e.g. reducing tissue plasminogen activator activity, thus reducing thrombogenic risk by decreasing platelet activation.¹⁰⁹

Diuretics and Beta-blockers

Diuretics (specifically thiazides) are mainstays of anti-hypertensive therapy, and although beta-blockers are now no longer first-line for hypertension, they remain widely prescribed in hypertensive patients, particularly in those with concomitant conditions such as angina. However, despite the efficacy of both classes of drugs in improving overall prognosis in hypertension, neither has been consistently shown to significantly improve endothelial function.^{73, 76, 110-113} One exception is nebivolol, a highly selective β_1 -blocker that has been shown to improve endothelial dysfunction both in vivo and vitro.^{114, 115} Dawes et al reported that acute intra-arterial administration of nebivolol improved forearm vasculature NO bioactivity in hypertensive patients. Although the mechanism by which nebivolol improves endothelial function in hypertension is not yet fully elucidated a putative pharmacological cross-reactivity between serotonin and β -receptors might potentially explain these vascular effects.^{116, 117} A possible antioxidant property of nebivolol has been suggested as an additional factor increasing NO-bioactivity or even a reduction in endothelin (ET-1) release.^{118, 119} The beneficial effects of nebivolol on the endothelium were confirmed in humans by Tzemos et al.¹²⁰ In a double-blind crossover trial, 12 hypertensive patients were given 8 weeks of treatment with bendroflumethiazide plus atenolol or nebivolol (and then switched to the other

group). With both treatments the blood pressure reduction was the same, however there was a significant increase in both forearm vasodilatory response to acetylcholine and the vasoconstrictor response to L-NMMA, suggesting that there may be a beneficial effect of nebivolol beyond blood pressure reduction. The endothelial effects of nebivolol are unique amongst beta-blockers and have been replicated in other studies.^{121, 122} Recent work by Vitale et al has suggested that nebivolol in combination with hydrochlorthiazide might at least be non-inferior to irbesartan with hydrochlorthiazide, perhaps giving some hope to beta-blockers in hypertension.¹²³ Indeed, a recent meta-analysis of 1,273 patients from 16 studies by Peller et al. on the effects of beta-blockers on endothelial function in various groups of patients did shown some benefit, however the study did include somewhat homogenous groups of patients.¹²⁴ The authors did find, consistent with studies in hypertension, that the best improvement in endothelial function was seen with 3rd generation beta-blockers such as nebivolol.

There have been some small studies suggesting that thiazide (and thiazide-like) diuretics might improve endothelial function. Some studies have shown that indapamide may also improve endothelial function in hypertensive patients when used in combination with ACE inhibitors.^{125, 126} Chlortalidone has also been shown to improve endothelial dysfunction in hypertensive patients.¹²⁷ The beneficial effects shown in these relatively small studies have yet to be confirmed in larger trials, particularly studies focussing on cardiovascular outcomes.

Endothelin Receptor Antagonists

Endothelin 1 (ET-1) acting through the ETA receptor is a potent vasoconstrictor that also promotes vascular smooth muscle cell proliferation.^{128, 129} Its expression in the vessel wall is promoted by ATII, and its vasoconstrictive effect appears to be enhanced in hypertensives.^{107, 130, 131} However, the plasma levels of ET-1 have not been consistently found to be raised in hypertension.^{132, 133} Nonetheless, ET-1 is released abluminally rather luminally, hence its plasma levels may not necessarily reflect its activity.¹³⁴ Increased ET-1 activity has been

found in salt-sensitive hypertension.^{135, 136} In human hypertension, Cardillo et al have shown that forearm intrabrachial administration of both selective and non-selective endothelin receptor blockers improved endothelium-dependent vasodilation.¹⁰⁸ The vasodilating properties of endothelin-1 receptor blockers might explain the beneficial effects on BP of bosentan, a non-selective ET-1 receptor blocker. Krum et al have shown that 4 weeks of treatment with bosentan reduced blood pressure as effectively as enalapril.¹³⁷ Moreover, the favourable effect of treatment with bosentan on blood pressure occurred without reflexive neurohormonal activation, which may suggest a more targeted effect of this drug on endothelial ET-1. Atrasentan (a selective ET-1 subtype A receptor antagonist) has also been shown to improve coronary endothelial function.¹³⁸

Future Directions and Conclusions

There is little doubt that endothelial dysfunction and hypertension co-exist, however many questions still need to be answered. While we know that reducing blood pressure improves prognosis, there is still a lot of doubt as to the incremental prognostic value of measurement of endothelial function. If this is established, we then need to find therapies that can improve endothelial function and whether this can translate to a longer-term prognostic benefit. The measurement of endothelial function itself needs to be standardized further and validated so that it can be used as both an endpoint in prognostic studies and as a marker in general clinical use. In common with much of the literature in this area, we have used the term “endothelial dysfunction” to refer exclusively to the vasodilatory properties of the endothelium, however it has numerous other functions, many of which could be quantified such as platelet aggregation and inflammation, and might have prognostic significance and may offer new therapeutic targets.

Endothelial dysfunction is associated with hypertension and its presence correlates with target organ damage. Furthermore, endothelial dysfunction may be both a cause and a consequence of hypertension. In this review, we have discussed the pathways by which endothelial

dysfunction may occur and the clinical evidence of its importance. Finally, we discussed the evidence looking at reversal of endothelial function, both by non-pharmacological methods and antihypertensive medications and therapies more directly targeted at the endothelium. Antihypertensive drug treatment improves and in some cases reverses endothelial dysfunction but this appear to depend very much on drug class and the vascular territories studied. Although calcium channel blockers, ACE-I, and ARBs seem to outperform beta-blockers (with the exception of nebivolol) and diuretics in terms of improving endothelial dysfunction, they do not seem to produce a greater antihypertensive effect. Most importantly, there is still some doubt within the scientific community as to whether the improvement in endothelial function is translated into long-term prognostic benefit.

In conclusion, although endothelial dysfunction is a conceptually attractive therapeutic target in hypertension, at this point in time, we lack convincing data that using endothelial dysfunction to guide our treatment would produce any better outcomes than using blood pressure targets to guide our treatment. This situation may change in the near future as more trials are conducted in this area.

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