Part I:

Pathophysiology

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Clinical Impact of Vasomotor Function Assessment and the Role of ACE-inhibitors and Statins

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***Vascul Pharmacol* 2005;42(3):125-40**

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Abstract

Impaired endothelial function is recognised as one of the earliest events of atherogenesis. Endothelium dependent vasomotion has been the principal method to assess endothelial function. In this article, we will discuss the clinical value of the different techniques to evaluate endothelium dependent vasomotion. To date, there seems not to be a simple and reliably endothelial function test to identify asymptomatic subjects at increased risk for cardiovascular disease in clinical practice. Recent studies indicate that pharmacological interventions, in particular with ACE-inhibitors and statins, might improve endothelial function. However, there is no solid evidence that improvement of endothelial function is a necessity for the observed reduction in cardiovascular events by these compounds. Overall, at this moment, there is no place in clinical practice for the use of endothelial function as a method for risk assessment or target of pharmacological interventions.

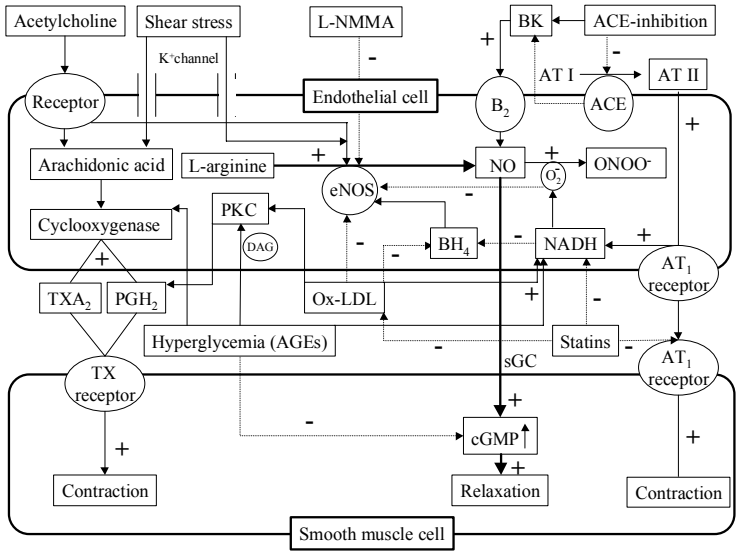
Introduction

Since the discovery of the obligatory role for the endothelium in relaxing arterial smooth muscles by acetylcholine in 1980[1], the endothelium has been the focus of intensive research. Currently, the endothelium is recognized to play a crucial role in vascular homeostasis in health and is considered to be early involved in the pathophysiology of cardiovascular disease[2-6]. The endothelium, a single cell layer of the vascular wall, has the ability to respond to physical, chemical, and neurohumoral stimuli by the production and release of a variety biological active substances, e.g. nitric oxide (NO), prostanoids, endothelin, angiotensin II, thrombomodulin, heparan sulphate, tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor (vWF), adhesion molecules and cytokines. NO, synthesized by the endothelial NO synthases, is the most investigated substance released from the endothelium and plays a pivotal role in endothelium-dependent vasodilatation and regulation of other protective functions of the endothelium. Functions of NO include regulation of vascular smooth muscle cell tonus and proliferation, blood hemostasis, vascular permeability, inflammatory response, platelet adherence and aggregation, and endothelial cell-leukocyte interaction[3;6;7]. In addition, the endothelium has organ-specific roles that are differentiated for various parts of the body, such as gas exchange in the lungs, control of myocardial function in the heart or phagocytosis in the liver and spleen[8]. A disturbance in the integrity or function of the endothelium is called endothelial dysfunction. In this review, we will focus on endothelium dependent vasodilatation as an measure of endothelial function (mediated predominantly by NO). We will discuss different methods of assessment, prognostic and clinical implications and pharmacological interventions aimed at lowering blood pressure and cholesterol, in particular with ACE-inhibitors and HMG-CoA reductase inhibitors, respectively.

Endothelial dependent vasomotor function

Endothelial cells synthesise NO through NO synthases (e.g. eNOS) by oxidation of the amino acid L-arginine (figure 1).[9] NO is thought to be the most important endothelial derived vasodilator. Required cofactors for eNOS functioning are haem, calmodulin, tetrahydrobiopterin, flavin mononucleotide and FAD, and NADPH[10-12]. NO is continuously produced by eNOS in the healthy endothelium in certain amounts in response to shear and pulsatile stretch of the vascular wall. The production of NO can be increased by many physiological stimuli, such as hypoxia, increase blood flow or shear stress, and pharmacological stimuli such as acetylcholine, bradykinine, adenosine triphosphate, adenosine diphosphate, thrombin, serotonin, histamine and substance P[13]. NO released from the endothelium in turn stimulates the soluble guanylate cyclase in the vascular smooth muscle cells, resulting in an increase in the formation of cyclic GMP[14-16]. Activation of cyclic GMP-dependent protein kinases alters the phosphorylation state of various proteins and cascades, subsequently resulting in the decrease in

Figure. 1 Effect of acetylcholine/shear stress, ACE-inhibition, and statins on vasomotor function.



BK: bradykinin, B2: B2-kinin receptor, ACE: angiotensin converting enzyme, AT: angiotensin, NO: nitric oxide, O2: superoxide anion, ONOO-: peroxynitrite anion, eNOS: endothelial Nitric oxide synthase, TXA2: thromboxane A2, PGH2: prostaglandin H2, PKC: protein kinase C, L-NMMA: L-NG-monomethyl arginine, BH4: tetrahydrobiopterin, NADH: NADH/NADPH oxidase, DAG: diacylglycerol, AGE: advanced glycation endproduct, ox-LDL: oxidised low-density lipoprotein, sGC: soluble guanylate cyclase, cGMP: cyclic guanosine monophosphate.
——— Upregulation, ····· Downregulation

vascular tonus through either a decrease in intracellular free calcium concentrations, calcium desensitization, and/or thin filament regulation, or a combination of these processes (see Refs[17;18] for reviews).
In addition to NO, other endothelial-derived factors contribute to vasorelaxation, e.g. prostacyclin and endothelium-derived hyperpolarizing factor (non-NO and non-prostaglandin mechanism). In contrast to NO, EDHF is predominantly present in smaller resistance arteries and less in large conduit vessels.[19] The relevance of non-NO dependent mechanisms is well established in animal and experimental models, but its clinical assessment and importance is still subject of investigation.

Assessment of endothelium dependent vasomotor function

Endothelium dependent vasodilatation has grown to be the read out of endothelial functioning based on the assumption that impaired endothelium dependent vasomotion reflects impaired NO production and other of its protective properties. In 1980, Furchgott and Zawadzki demonstrated that relaxation of isolated rabbit thoracic aorta and other blood vessels by acetylcholine was dependent on the presence of endothelium[1]. In the absence of

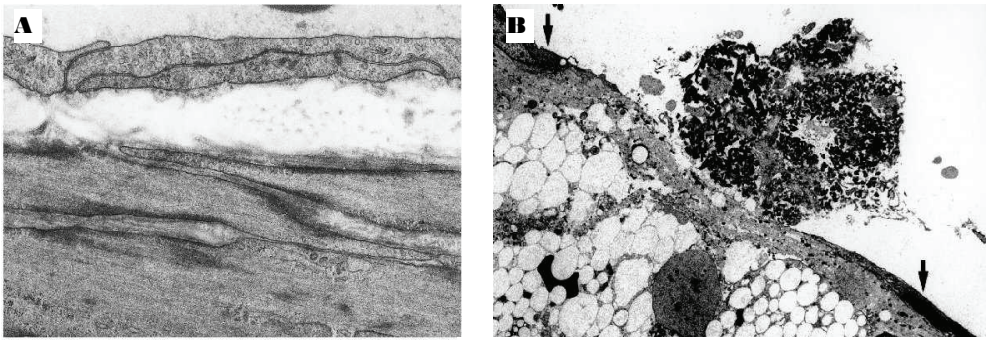
endothelium acetylcholine caused vasoconstriction through a direct effect on the muscarinic receptors of the vascular smooth muscle cells. Similar experiments confirmed the presence of endothelium dependent vasomotor function in human isolated internal mammary arteries, a vessel widely used for coronary bypass surgery[20]. It has been suggested that the decreased endothelium dependent vasorelaxation in saphenous venous segments to acetylcholine, compared to mammary artery segments, can explain the differences in graft patency after bypass[21-24]. In isolated human coronary arteries, obtained from hearts of cardiac transplant patients, the endothelium dependent vasomotor function in response to substance P, bradykinin, and Ca^{+}-ionophore A23187 of atherosclerotic arteries was attenuated[25].

Abnormal vasomotor response to acetylcholine can also be demonstrated in vivo in the catheter laboratory using quantitative angiography. In patients with coronary atherosclerosis in the left anterior descending coronary artery, acetylcholine induced vasoconstriction[26]. In subsequent studies, progressive impairment of endothelial dependent vasomotor function was demonstrated in coronary arteries of patients with different early stages of atherosclerosis [27;28] and traditional risk factors[29-33].

However, coronary endothelial testing during catheterization is invasive, not without risk, and time consuming[34]. Assessment of endothelial function in the brachial artery could be a solution to some of these limitations. Endothelial dependent vasomotor function can be assessed in peripheral arteries by using intra-arterial infusions of pharmacologic stimuli, like acetylcholine, to enhance NO release. During venous occlusion strain gauge plethysmography total forearm blood flow (FBF) and thus vasomotor function can be calculated by measuring increase in forearm volume over time after infusion of acetylcholine[35-38]. Indeed, impairment of endothelial function as assessed by FBF is also associated with cardiovascular risk factors, including hypercholesterolaemia, diabetes mellitus and cigarette smoking[39-41]. In addition, we found a significant correlation between the endothelium dependent vasodilatation in the coronary and brachial artery after acetylcholine infusion in patients referred for a first diagnostic angiogram[42]. Venous occlusion plethysmography in the brachial artery consists of less risk compared to assessment of the coronary artery, but is still not suitable in asymptomatic patients due to its invasive approach.

Since impaired NO release in humans can be demonstrated by an impaired vasomotor response to eNOS activation, it can also be demonstrated by physiological stimuli, such as exercise-[43] or cold-pressor testing[27;44]. Another physiological stimulus, blood flow, allows non-invasive assessment of endothelial dependent vasodilatation. Flow-mediated vasodilatation (FMD) in the brachial artery measures the diameter of the brachial artery at rest and during reactive hyperaemia with high-resolution ultrasound and has been widely used for quantifying endothelium dependent vasodilatation[45;46]. Anderson et al.[47] demonstrated a relation between coronary artery endothelium-dependent vasomotor responses to acetylcholine and the FMD. There was a positive predictive value of 95% between an abnormal brachial artery dilatation and coronary endothelial dysfunction. However, the sensitivity for detecting coronary endothelial dysfunction was only 49%. Besides quantitative angiography, FBF and FMD, there are some other methods, such as positron emission

Figure 2



A; Coronary artery without obstructive coronary artery disease and an intact endothelial layer. B; Coronary artery with a microthrombus centrally located, the endothelium is damaged or obliterated at the site of injury. The black arrows indicate adjacent endothelial cells. Reproduced with permission of AC van der Wall (Academic Medical Center, Amsterdam).

tomography[48] and transthoracic doppler echocardiograpy[49;50], but these are currently not commonly used and only small sample sizes have been reported. To date, non-invasive assessment of the flow-mediated vasodilatation with ultrasound seems to be the most attractive method to assess endothelium dependent vasodilatation because of its safety and the potential to measure vascular function repeatedly over time.

Prognostic value of endothelium dependent vasomotor function

To our knowledge, no studies have been published on the prognostic value of endothelial dependent vasodilation of isolated human vascular segments. However, several studies (table 1) have investigated the prognostic value of endothelium dependent vasodilatation in the coronary artery as assessed at the catheter laboratory. Suwaidi et al.[51] were the first to report that endothelial dysfunction is associated with increased cardiac events in 157 patients with mild coronary artery disease (CAD) during a 2.3-year follow-up. This finding was confirmed by Schächinger et al.[52] who studied 147 patients with mild CAD during a median follow-up of 7.7 years. Since these two studies had a relatively small number of events (6 and 16, respectively) it was not considered as sufficiently convincing evidence. However, Halcox et al.[53] provided similar data, retrospectively, about the predictive value of coronary endothelial dependent function in a study in 308 patients with mild to moderate CAD and even in patients with angiographically normal coronary arteries, with a follow up of 46 months (35 events). Another retrospective study demonstrated an independent association between coronary endothelial function and cerebrovascular events in patients without obstructive coronary artery disease at an early stage of

Table 1. Prognostic studies of coronary and peripheral endothelial function

Study	N	Extent of CAD	Design	Method	Cut-off value	Events, n	Follow-up, months	Result
Suwaidi [51]	157	< 40% stenosis	prospective	CEDV	-20 %	6	28	+
Schachinger [52]	147	1 VD	prospective	CEDV	0 %	16	92	+
Hollenberg [165]	73	< 50% stenosis	prospective	CEDV	5 %	14	9	+
Halcox [53]	308	Mild to moderate	retrospective	CEDV	0 %	35	46	+
Targonski [54]	503	< 30 % stenosis	retrospective	CEDV	20 %	25	16	+
von Mering [55]	163	75 % normal or mild VD	prospective	CEDV	0 %*	58	48	+
Asselbergs [166]	277	0-3 VD	prospective	CEDV	0 %	24	47	-
Schindler [57]	130	0 VD	prospective	CPT	0 %	26	45	+
Nitenberg [58]	128	0 VD	retrospective	CPT	0 %	27	45	+
Perticone [59]	225	Unknown	prospective	FBF	Tertiles	29	32	+
Heitzer [60]	281	1-3 VD	prospective	FBF	Median	91	54	+
Neunteufl [67]	73	0-3 VD	prospective	FMD	10 %	27	12	+
Gokce [69]	187	Unknown	Prospective	FMD	Tertiles	45	1	+
Gokce [68]	199	Unknown	prospective	FMD	Tertiles	35	14	+
Chan [167]	152	Unknown	prospective	FMD	FMD/NMD ratio	22	34	+
Brevetti [168]	131	Unknown	prospective	FMD	median	39	23	+
Fathi[169]	444	Unknown	prospective	FMD	Tertiles	49	24	-

CAD: coronary artery disease, CEDV: coronary endothelial dependent vasodilatation, CPT: cold-pressor testing, FMD: flow mediated vasodilatation, NMD: nitroglycerin mediated dilation. *In the multivariate analysis, no cut-off value was used, but the change in diameter after acetylcholine infusion was continuously entered into the model.

atherosclerosis[54]. More recently, the Women's Ischemia Syndrome Evaluation (WISE) Study investigated the prognostic value of coronary vascular dysfunction in 163 women referred for diagnostic angiogram for evaluating myocardial ischemia[55]. Seventy-five percent had no or mild coronary artery disease and the degree of coronary artery disease and the change in diameter after intracoronary acetylcholine infusion were both predictive for cardiovascular events. Unfortunately, it is unclear how many events occurred in the group with mild and in the group with severe coronary artery disease. Fifty-eight (36%) of these so-called lower-risk women had a cardiovascular event during a follow-up period of 48 months and the WISE study has thereby the highest event rate of all studies listed in table 1. We studied 277 patients referred for a first coronary angiogram[56]. In contrast to other studies, we did not exclude patients with advanced atherosclerosis who were candidates for revascularization. This might explain why we were unable to demonstrate prognostic value of coronary endothelium dependent vasomotor function. Furthermore, discontinuing vasoactive medication before the coronary angiogram in this study may confounded this finding. It might have been the case that non-responders to cardioprotective medication experienced events. Unfortunatly, how previous studies dealt with vasoactive medication prior to endothelium assessment is not clear. Two other studies reported that endothelial vasomotor function of the coronary artery in response to cold-pressor testing have prognostic value in patients with normal coronary angiograms[57,58]. Collectively, the above studies indicate that coronary vasomotor function in patients with an increased cardiovascular risk profile can predict future cardiovascular events. However,

measuring endothelial function in coronary arteries is only justifiable in high-risk patients and therefore does not add any value to primary prevention programs.
Only recently, the prognostic value of peripheral assessment of endothelial dependent vasodilatation by FBF in response to intra-arterial acetylcholine infusion was demonstrated[59;60]. In patients with initially untreated and uncomplicated essential hypertension and in patients with documented coronary artery disease forearm endothelial dysfunction appeared a marker for future cardiovascular events[59;60]. Non-invasive assessment of peripheral endothelial dependent vasodilatation by FMD might also be a marker for increased cardiovascular risk[61]. Two studies have reported a significant (modest-strong) relation with impaired endothelial dependent relaxation in the coronary arteries and brachial arteries[62;63]. However, the results of FBF and FMD may not be analogous[42;64] and reproducibility of FMD can be poor[42;65]. Neunteufl et al.[66] demonstrated that FMD measurements are independently associated with the angiographic extent of CAD. Interestingly, the same group found a relation between endothelial function measured by the FMD and need for cardiac revascularization[67]. Gokce et al. demonstrated in a prospective study that an impaired FMD predicts short-term and long-term cardiovascular events after vascular surgery[68;69]. In another report it was shown that functional integrity following arterial bypass surgery was preserved, which may partially explain the long term beneficial outcome after arterial graft surgery[70]. However, in a recent study, involving a relatively large number of patients (n=444) at relatively high risk, Fathi et al, investigated whether FMD measurements have an additional value in predicting mortality and cardiovascular morbidity relative to other predictors of outcome[71]. Although they found in univariate analysis that the most disturbance of FMD had greater subsequent cardiac morbidity and mortality than those with normal or mildly abnormal FMD, in multivariate analysis this could be independently predicted by intima medial thickness (IMT) and LV mass rather than FMD. Only in a subgroup undergoing stress testing, FMD provided additional information (along with IMT and LV mass).
When considering the above, it should be noted that studies demonstrating prognostic value of coronary vasodilation have predominantly been performed in patients with mild to moderate CAD. In patients with more severe CAD, peripheral endothelial function testing was used to investigate its predictive value. This discrepancy in study designs may suggest that endothelium dependent vasodilatation test for assessment of endothelium function can only be assessed in vessels with intact endothelium, e.g. the brachial or coronary artery with mild atherosclerosis. To visualise this conception, figure 2a shows a coronary artery without obstructive coronary artery disease and an intact endothelial layer. In contrary to mild coronary artery disease, the endothelium is damaged or obliterated in coronary arteries with severe atherosclerosis and plaques (figure 2b). Therefore, we hypothesize that coronary endothelial function testing might not be a powerful diagnostic modality in patients with advanced atherosclerosis. In addition, use or non-use of vasoactive mediation introduces an important potential confounder since non-responders to vasoactive medication might continue to have diminished endothelium vasomotor function in contrast to responders. In this respect, discontinuing medication might provide a better information of the intrinsic health of the endothelium, while continuing medication probably provides better information

of the endothelium function in 'real life', when events do occur. Which of these two situations provides the strongest prognostic information still remains to be elucidated.
In summary, most of the aforementioned studies demonstrate a predictive value of endothelial function in patients independent of traditional cardiovascular risk factors. Whether or not this is truly independent or additive, in all situations remains to be established. Furthermore, most studies were retrospective in nature, with few events and performed in selected patient populations only. Invasive endothelial function measured by intracoronary or intrabrachial acetylcholine infusion does not seem to have future as primary prevention tool considering their risk and costs. Nevertheless, these tests continue to have value for academic reasoning and assessing vascular response to therapeutic intervention over time in symptomatic or high-risk patients. The FMD assessment of endothelial dependent vasomotor function seems to be the most attractive candidate since it can safely be applied to large and asymptomatic patients. However, disadvantages of FMD are the high variability, its modest association with coronary endothelial function and lack of standardisation between centres.

Intervention on endothelium dependent vasomotor function

Numerous studies have reported the beneficial effects of several physiological and pharmacological interventions on endothelial function[72-81]. Among these, ACE-inhibitors and HMG CoA reductase inhibitors (statins) are most extensively investigated in experimental and clinical setting. Both drugs have repeatedly been demonstrated to be very effective in decreasing cardiovascular events in high-risk populations. Therefore, this review is limited to the effects of these drugs on endothelial function.

Hypertension, ACE-inhibitors and endothelium dependent vasomotor function

It has been repeatedly shown that hypertension impairs endothelium dependent vasomotor function in animal models as well as human studies (for reviews see[82;83] Impairment of endothelium dependent vasodilatation has been extensively documented in hypertensive subjects with several methods and in different arteries[84-90]. It has not yet been fully elucidated whether impaired endothelium dependent vasodilatation causes hypertension or vice versa. Nevertheless, antihypertensive treatment with ACE-inhibitors, calcium-channel blockers, angiotensin 2 receptor blockers, betablockers and diuretics can improve endothelium dependent vasodilatation in animal experimental models of hypertension[91-96]. In humans, it has also been demonstrated that improvement in endothelial function may be obtained after antihypertensive therapy and, in addition, clearly identifies patients who possibly have more favourable prognosis[97]. The most potent improvement of endothelium function might be obtained by ACE-inhibitors. ACE inhibitors not only prevent the production of angiotensin II from angiotensin I, but also stimulates the production of

Table 2. Human studies on the effect of ACE-inhibitors on endothelial function

Study	Patient characteristics	N	Method	ACE-inhibitor	Follow-up	Result
Mancini [170]	CAD	105	CEDV	Quinapril	6 months	+
Prasad [171]	Coronary sclerosis or risk factors	56	CEDV	Enalapril	Immediately after infusion	+
Antony [100]	Hypertension	10	CPT	Perindopril	Immediately after infusion	+
Hirooka [172]	Hypertension	12	FBF	Captopril	Acute	+
Kiowski [173]	Hypertension	10	FBF	Cilazapril	5 months	-
Creager [174]	Hypertension	44	FBF	captopril enalapril	7-8 weeks	- -
Iwatsubo [175]	Hypertension	26	FBF	Temocapril	6 months	+
O'Driscoll [176]	Type I DM	9	FBF	enalaprilat / enalapril	Acute infusion / 1 month	+ / +
Haefeli [177]	Healthy volunteers	44	FBF	quinapril enalapril	Immediately after infusion	+ -
Taddei [107]	Hypertension	20	FBF	Lisinopril	6-8 hours / 1 month / 12 months	+/+
Millgard J [178]	Hypertension	23 / 5	FBF	Captopril	1 hour / 3 months	+
O'Driscoll[179]	Type II DM	10	FBF	Enalapril	4 weeks	+
Benacerraf [104]	CAD	26	FBF	Quinapril	Immediately after infusion	-
Lee [108]	Hyperlipidaemia	40	FBF	Lisinopril	6 months	+
Houben [180]	Hypertension	12	FBF	quinapril enalapril	Immediately after infusion	+ -
Higashi [181]	Hypertension	296	FBF	any ACEi	> 24 weeks	+
Butler [182]	Smokers	23	FBF	Lisinopril	8 weeks	+
Nagy [105]	Hypertension	21	FMD	Benazepril	2 hours/ 1 month	- / -
Mullen [183]et al. (1998)	Type 1 DM	91	FMD	Enalapril	12 / 24 months	- / -
Hornig [184]	CHF	15	FMD	quinapril enalapril	Immediately after L-NMMA	+ -
Wilmink [185]	Healthy volunteers	30	FMD	Quinapril	2 weeks	+
McFarlane [186]	Type 1 DM	20	FMD	Perindopril	12 weeks	-
Arcaro G, [187]	Microalbuminuric type 1 DM	9	FMD	captopril / enalapril	1 week / 1 week	+/+
Anderson [103]et al. (2000)	CAD	80	FMD	quinapril enalapril	8 weeks	+ -
Esper [188]	CAD with hypercholesterolemia	38	FMD	Enalapril	8 weeks	+
Bae [106]	CAD	39	FMD	Lisinopril	2 hours	-
Bots [189]	coronary artery disease	345	FMD	Perindopril	3 years	?*

EF: endothelial function, CAD: coronary artery disease, CHF: chronic heart failure, DM: diabetes mellitus, FBF: Forearm Blood Flow assessed by venous occlusion plethysmography, FMD: endothelium dependent Flow-mediated vasodilatation of the brachial artery, CPT: cold-pressor test, CEDV: coronary endothelium dependent vasodilatation after infusion of acetylcholine, L-NMMA: L-NG-monomethyl arginine, ACEi: ACE-inhibitor. * Not completed.

bradykinin. Bradykinin contributes to the effects of ACE inhibitors in clinical setting[98]. Several pathways are activated by both actions, which are illustrated in figure 1. Indeed, treatment with ACE-inhibitors has been shown to improve endothelial function in humans (table 2)[99-102]. The Brachial Artery Normalisation of Forearm Function (BANFF) study was designed to compare the effect of ACE-

inhibition with quinapril or enalapril, angiotensin II blockade with losartan, and calcium-channel blocking therapy with amlodipine on FMD in 80 patients with CAD[103]. The authors demonstrated that only quinapril improved FMD after eight weeks of treatment in patients with CAD. These results were in concordance with other studies comparing enalapril and quinapril. Moreover, the beneficial effect of quinapril was confirmed in studies using FMD, FBF, and coronary endothelial function (table 2). Only Benaceraf[104] et al. did not demonstrate a beneficial effect of quinapril in patients with CAD. Nevertheless, quinapril improved FBF in healthy subjects in this study. The discrepancy found in the BANFF study between quinapril and losartan suggest an important role of kinins in endothelium dependent vasodilatation, as losartan did not augment FMD. However, despite extensive experimental evidence and the studies using quinapril, the beneficial effects of other ACE-inhibitors on endothelial function in humans are more inconsistent (table 2). Benazepril only augmented FMD in hypertensive patients with a FMD <5% at baseline[105]. Lisinopril showed an antioxidant effect, but showed no acute benefit in terms of endothelial function in patients with CAD[106]. In addition, lisinopril increased vasodilatation in response to bradykinin, but not to acetylcholine in hypertensive patients[107]. On the other hand, two studies showed that lisinopril increases FBF in cigarette smokers and hyperlipidaemic subjects[108;109]. Other ACE-inhibitors than enalapril and quinapril are more difficult to evaluate, because only a few human studies have been performed with those drugs, such as benazepril, captopril, perindopril, cilazapril, ramipril and temocapril (table 2).
In the near future, the results of a nested substudy of the EUROPA trial, studying the effects of perindopril on stable coronary artery disease, will be published[110]. Using FMD measurements the effect 3 years of perindopril will be assessed in a relatively large cohort of patients (n=345). The prospective findings of this study may further enhance our understanding and provide a more definite answer on effects of ACE inhibitors on endothelium functioning.

Effects of cholesterol lowering therapy and HMG CoA reductase inhibitors on endothelium dependent vasomotor function

Effects of hypercholesterolemia on endothelial function have also been extensively studied. Numerous studies indicate cholesterol to be an independent predictor for endothelial dysfunction and to have an inverse correlations with endothelium dependent vasodilatation with different methods of assessment and in different arteries[29;45;111-117]. Several explanations can account for the decreased endothelial function in hypercholesterolemia. (Oxidized) LDL cholesterol can decrease eNOS activity at several levels, such as downregulation mRNA[118], inhibition of membrane signal transduction[119] and NO inactivation[120]. In addition, increased vascular oxidative stress is associated with hypercholesterolemia and could further diminish the availability of NO[121]. Evidence for causality in humans was provided by several LDL apheresis studies. A single LDL apheresis can result in LDL reductions of 40-90% within 3 hours. In hypercholesterolemia patients improvement of endothelial dependent vasodilatation could be demonstrated directly after LDL apheresis in the coronary artery[122;123] and forearm vessel (FBF)[124]. A reduction of triglycerides

showed no such correlation. This studies strongly supports the concept that hypercholesterolemia in itself causes endothelial dysfunction that can be immediately reversed by an acute aggressive reduction of serum cholesterol.
Only very few studies investigated pharmacological interventions on endothelium dependent vasorelaxation not using statins. Treatment with cholestyramine in hypercholesterolaemic patients resulted in a 29% reduction in total cholesterol after 6 months and a significant improvement of acetylcholine induced vasomotor response of the coronary artery[125]. Treatment with gemfibrozil resulted in improvement in 10 subjects with DM, but had no effect in 100 subjects with LDL <160mg/dl[126;127]. Most studies used statin treatment as cholesterol lowering intervention. Statin trials were able to confirm beneficial effects of statins on endothelial vasomotor function in hypercholesterolemia (table 3). However, besides cholesterol lowering efficacy, evidence is accumulating statins might have additional effects on the endothelium (for reviews see[128-131]). In vitro, statins can increase eNOS mRNA expression, stability as well as eNOS activity[132;133]. Furthermore, protection of statins was completely abolished in eNOS knockout mice and after inhibition of eNOS with L-NAME[134;135]. Considering these cholesterol-independent mechanisms of action, statin studies on endothelium dependent effects were further evaluated. Laufs et al, demonstrated that high dose atorvastatin treatment in healthy normocholesterolemic subjects with normal vascular function increases endothelium-dependent FBF within 24 hours, before serum cholesterol and hs-CRP which decreased after 2 days of treatment[136]. Furthermore, withdrawal of atorvastatin therapy acutely impaired vascular function independent of the sustained decreased cholesterol levels. This acute effect was confirmed by Omori et al. using cerivastatin[137]. A single dose of cerivastatin increased vascular endothelial responsiveness transiently in cerivastatin treated subjects after 3 hours, also before an effect on serum cholesterol levels was detectable. The time course of cholesterol lowering at the beginning and end of treatment disassociated from alterations of vascular function. Therefore, these data support the existence of cholesterol-independent effects of statins in humans. Similar, pravastatin improved endothelial dependent vasodilatation after 3 days of treatment in normocholesterolemic CAD patients[138]. This improvement in endothelial function was completely blocked by coinfusion of the NO synthase inhibitor L-NMMA, thus seems to be attributable to a potentiation of eNOS activity. After menopause, most healthy women show an impairment of peripheral vasodilatation. 10 days of atorvastatin treatment improved endothelium-dependent vasodilatation in postmenopausal normocholesterolemic women, without affecting serum lipoproteins. Furthermore, the effect was potentiated by L-arginine and blunted by L-NMMA, strongly suggesting an association with increased NO production by statin treatment[139]. Recently, it was demonstrated that impaired endothelial function caused by cigarette smoking could be improved by statin treatment in normocholesterolemic smokers, without associations between baseline lipid levels and change in lipid levels[140]. These studies all suggests that statins probably exerts a direct action on the arterial endothelium independently of (serum) cholesterol levels. However, in diabetic subjects results are less consistent (table 3).[141-147] In diabetic studies, endothelial function seems not to be associated with an traditional lipid variable,

Table 3. Human studies on the effect of statins on endothelial function

Study	Patient characteristics	n	Method	Statin	Follow-up	Result
Egashira [190]	hypercholesterolemia	9	CEDV	pravastatin	6 months	+
Treasure [191]	CAD	23	CEDV	lovastatin	12 days / 5.5 months	- / +
Anderson [62]	Cholesterol (4.7-7.2 mmol/l)	49	CEDV	lovastatin cholestyramide probucol	1 year	+
Houghton [192]	Normal coronary arteries	6	CEDV	pravastatin	6 months	-
Vita [153]	CAD	60	CEDV	simvastatin	6 months	-
Penny [193]	Hypercholesterolemia	29	CEDV	lovastatin	18 months	+*
ENCORE [154]	CAD	343	CEDV	cerivastatin	6 months	-
Wassmann [194]	Angina pectoris	27	CEDV	pravastatin	24 hours	+
O'Driscoll [195]	Cholesterol (6.0-10.0 mmol/l)	10	FBF	simvastatin	4 weeks / 3 months	+ / +
Perticone [196]	hypercholesterolemia	40	FBF	atorvastatin	1 month	+
John [197]	hypercholesterolemia	37	FBF	cerivastatin	2 weeks	+
Duffy [144]	hypercholesterolemia	26	FBF	simvastatin	After L-NMMA	-
van de Ree [143]	Type 2 DM	17	FBF	simvastatin	6 weeks	-
Laufs [136]	Healthy male	28	FBF	atorvastatin	48 hours	+
Mercuro [139]	Postmenopausal women	28	FBF	atorvastatin	10 days	+
van Etten [145]	Type 2 DM	44	FBF	atorvastatin	4 weeks	-
Wassmann [198]	Normocholesterolemic	18	FBF	atorvastatin	6 weeks	+
Vogel [148]	Hypercholesterolemia	7	FMD	simvastatin	12 weeks	+
Neunteufl [199]	hypercholesterolemic	7	FMD	simvastatin vitamin E	20 weeks	+
Simons [200]	hypercholesterolemia	32	FMD	atorvastatin simvastatin + cholestyramine	30 weeks	+ +
Sheu [141]	Type 2 DM with hypercholesterolemia	21	FMD	simvastatin	24 weeks	-
Dupuis [201]	ACS	60	FMD	pravastatin	6 weeks	+
Jarvisalo [202]	CAD	45	FMD	statins	3 months	+
Rashid [203]	CAD	8	FMD	simvastatin	3 / 6 months	+ / +
Mullen[204]	Type 1 DM	84	FMD	atorvastatin	6 weeks	+
Tsunekawa[152]	Type 2 DM	27	FMD	cerivastatin	3 days 3 months	+
Sheu [142]	Type 2 DM with hypercholesterolemia	12	FMD	simvastatin	12 weeks	+
Malik [205]	hyperlipidemia	29	FMD	fenofibrate atorvastatin	10 weeks	- -
Stein [206]	hypercholesterolemia	37 17	FMD	pravastatin simvastatin	6 months	- -
Van Venrooij [207]	Type 2 diabetes	133	FMD	atorvastatin	30 weeks	-
Dogra [208]	Nephrotic syndrome	10	FMD	any statin	12 weeks	+
Omori [209]	Healthy volunteers	30	FMD	cerivastatin	1h/3h/6h/12h	-/+/-/-
de Jongh [210]	FH	69	FMD	simvastatin	28 weeks	+
Vita[211]	CAD	51	FMD	Atorvastatin	36 hours	-
van Haelst [212]	FH	35	FMD	simvastatin	6 weeks	-
Economides [213]	Diabetes Type 1/2	40	FMD	atorvastatin	12 weeks	+
Beckman [214]	Cigarette smokers Healthy controls	20 20	FMD	atorvastatin	4 weeks	+ -

EF: endothelial function, CAD: coronary artery disease, CHF: chronic heart failure, DM: diabetes mellitus, FBF: Forearm Blood Flow assessed by venous occlusion plethysmography, FMD: endothelium dependent Flow-mediated vasodilatation of the brachial artery, CEDV: coronary endothelium dependent vasodilatation after infusion of acetylcholine, ACS: acute coronary syndromes, FH: familial hypercholesterolemia, L-NMMA: L-NMMA: L-N^G-monomethyl arginine. *Improvement only in the most constricted segments

in contrast to studies of nondiabetic subjects[148-151]. Interestingly, in elderly type 2 diabetic patients 3 days of cerivastatin treatment did not result in change in lipid concentrations, but did improve the FMD and this was associated with an increase in plasma NOx and decrease of oxidant markers[152]. Thus, whether in diabetic subjects, hyperglycemia is the principle cause of endothelial dysfunction resistant to statin therapy remains to be further elucidated.
In contrast to several studies investigating peripheral endothelial function in normocholesterolemic patients, two placebo-controlled randomised studies failed to show an improvement on coronary endothelial function after 6 months of treatment with simvastatin and cerivastatin, the CARATS and the ENCORE study[153;154]. The improvement of coronary endothelial function in the placebo group might have resulted in the regression to the mean phenomenon. Both groups assessed the effects on the most constrictive segment. However, when all coronary segments were analysed, this phenomenon disappeared. Taken all together, more positive than negative studies have been published for each method of endothelial function testing (table 3). The currently available studies strongly suggest that treatment of hypercholesterolemia can restore endothelial dysfunction. At the moment, conflicting clinical evidence of statin therapy exists on endothelium dependent effects beyond cholesterol lowering. Furthermore, no study has demonstrated whether improvement of endothelium dependent vasodilatation is a necessity for the efficacy of statin treatment.

Clinical Implications of therapeutic interventions on endothelial dependent vasomotor function

Improvement of endothelial function can be induced by statin therapy and possible ACE inhibitors. Like aspirin[73;75;155], statins and ACE inhibitors definitely improve cardiovascular prognosis concomitantly with endothelial dependent vasomotor function. However, endothelial function can also be improved with other pharmacological interventions, such as vitamins/antioxidants[78;156-158] and hormone replacement therapy[79;80;159] but without positive effects on clinical outcomes[160-164]. Thus, improvement of endothelial function is not necessarily a good surrogate marker of risk reduction. The clinical value of persistent endothelial dysfunction after initiation of a well proven treatment has not been well established. Currently, only one study reported the lack of change in FMD in response to blood pressure lowering therapy could predict cardiovascular events in 400 postmenopausal hypertensive women[97].

Conclusions

In this paper, we reviewed the most commonly used methods to assess endothelial dependent vasomotor function. It is conceivable to assume that endothelial dependent vasodilatation is of prognostic value for future cardiovascular events. Non-invasive assessment of the flow-mediated vasodilatation with ultrasound seems to be the most attractive method to assess endothelium dependent vasodilatation in low-risk and asymptomatic patients because of its safety and the potential to measure vascular function repeatedly. However, studies aimed at the prognostic value were only performed in selected

patients referred for evaluation of cardiovascular disease. Consequently, these data cannot be extrapolated to the general population. In addition, the assessment of flow-mediated dilatation is a sophisticated and expensive procedure, which may only be useful for academic reasoning towards new diagnostic and treatment modalities.
Secondly, we discussed blood pressure and cholesterol lowering interventions, in particular with ACE inhibitors and statins, on endothelium dependent vasodilatation, which seems beneficial in small sample sizes. Currently, large randomised population based prospective studies on treatment of endothelial function with statins or ACE inhibitors in low risk patients are lacking. Improvement of endothelial dependent vasodilatation could, theoretically, exert a beneficial effect on clinical outcome. However, several interventions improving endothelial function did not improve clinical outcome and therefore it remains questionable whether improvement of endothelial function is a necessity to improvement in cardiovascular prognosis.

Acknowledgements

The authors are indebted to Dr. A.C. van der Wall for providing figure 2. We gratefully acknowledge Dr. J.F. May for contributing substantially to the initiation of this review. F.W. Asselbergs is a research fellow of the Netherlands Heart Foundation (2003T010). P. van der Harst is supported by ZonMW (920-03-236) of The Netherlands Organization for Health Research and Development.

References

1. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373-76.
2. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993;329:2002-12.
3. Rubanyi GM. The role of endothelium in cardiovascular homeostasis and diseases. J Cardiovasc Pharmacol 1993;22 Suppl 4:S1-14.
4. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999;340:115-26.
5. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation 2004;109:III27-III32.
6. Glasser SP, Selwyn AP, Ganz P. Atherosclerosis: risk factors and the vascular endothelium. Am Heart J 1996;131:379-84.
7. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993;329:2002-12.
8. Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med 1990;323:27-36.
9. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988;333:664-66.
10. Marletta MA. Nitric oxide synthase structure and mechanism. J Biol Chem 1993;268:12231-34.
11. Fleming I, Busse R. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. Am J Physiol Regul Integr Comp Physiol 2003;284:R1-12.
12. Albrecht EW, Stegeman CA, Heeringa P, Henning RH, van Goor H. Protective role of endothelial nitric oxide synthase. J Pathol 2003;199:8-17.
13. Luscher TF, Vanhoutte PM. The Endothelium Modulator of Cardiovascular Function. Boca Raton, Fla: CRC Press, 1990:1-215.
14. Rapoport RM, Murad F. Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. Circ Res 1983;52:352-57.
15. Rapoport RM, Draznin MB, Murad F. Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. Nature 1983;306:174-76.
16. Draznin MB, Rapoport RM, Murad F. Myosin light chain phosphorylation in contraction and relaxation of intact rat thoracic aorta. Int J Biochem 1986;18:917-28.
17. Lincoln TM, Dey N, Sellak H. Invited review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: from the regulation of tone to gene expression. J Appl Physiol 2001;91:1421-30.
18. Hofmann F, Ammendola A, Schlossmann J. Rising behind NO: cGMP-dependent protein kinases. J Cell Sci 2000;113 (Pt 10):1671-76.
19. McGuire JJ, Ding H, Triggle CR. Endothelium-derived relaxing factors: a focus on endothelium-derived hyperpolarizing factor(s). Can J Physiol Pharmacol 2001;79:443-70.
20. Schoeffter P, Dion R, Godfraind T. Modulatory role of the vascular endothelium in the contractility of human isolated internal mammary artery. Br J Pharmacol 1988;95:531-43.

21. Luscher TF, Diederich D, Siebenmann R, Lehmann K, Stulz P, von Segesser L, Yang ZH, Turina M, Gradel E, Weber E, . Difference between endothelium-dependent relaxation in arterial and in venous coronary bypass grafts. N Engl J Med 1988;319:462-67.
22. Yang ZH, Diederich D, Schneider K, Siebenmann R, Stulz P, von Segesser L, Turina M, Buhler FR, Luscher TF. Endothelium-derived relaxing factor and protection against contractions induced by histamine and serotonin in the human internal mammary artery and in the saphenous vein. Circulation 1989;80:1041-48.
23. Werner GS, Wiegand V, Kreuzer H. Effect of acetylcholine on arterial and venous grafts and coronary arteries in patients with coronary artery disease. Eur Heart J 1990;11:127-37.
24. Tadjkarimi S, O'Neil GS, Luu TN, Allen SP, Schyns CJ, Chester AH, Yacoub MH. Comparison of cyclic GMP in human internal mammary artery and saphenous vein: implications for coronary artery bypass graft patency. Cardiovasc Res 1992;26:297-300.
25. Forstermann U, Mugge A, Alheid U, Haverich A, Frolich JC. Selective attenuation of endothelium-mediated vasodilation in atherosclerotic human coronary arteries. Circ Res 1988;62:185-90.
26. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046-51.
27. Zeiher AM, Drexler H, Wollschlaeger H, Saurbier B, Just H. Coronary vasomotion in response to sympathetic stimulation in humans: importance of the functional integrity of the endothelium. J Am Coll Cardiol 1989;14:1181-90.
28. Zeiher AM, Drexler H, Wollschlager H, Just H. Modulation of coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. Circulation 1991;83:391-401.
29. Vita JA, Treasure CB, Nabel EG, McLenachan JM, Fish RD, Yeung AC, Vekshtein VI, Selwyn AP, Ganz P. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1990;81:491-97.
30. Zeiher AM, Drexler H, Wollschlager H, Just H. Endothelial dysfunction of the coronary microvasculature is associated with coronary blood flow regulation in patients with early atherosclerosis. Circulation 1991;84:1984-92.
31. Antony I, Aptecar E, Lerebours G, Nitenberg A. Coronary artery constriction caused by the cold pressor test in human hypertension. Hypertension 1994;24:212-19.
32. Nitenberg A, Antony I, Aptecar E, Arnoult F, Lerebours G. Impairment of flow-dependent coronary dilation in hypertensive patients. Demonstration by cold pressor test induced flow velocity increase. Am J Hypertens 1995;8:13S-8S.
33. Quyyumi AA, Dakak N, Andrews NP, Husain S, Arora S, Gilligan DM, Panza JA, Cannon RO, III. Nitric oxide activity in the human coronary circulation. Impact of risk factors for coronary atherosclerosis. J Clin Invest 1995;95:1747-55.
34. Tio RA, Monnink SH, Amoroso G, Jessurun GA, Veeger N, Volkers C, Hautvast R, Tan ES, van Gilst WH, van Boven AJ. Safety evaluation of routine intracoronary acetylcholine infusion in patients undergoing a first diagnostic coronary angiogram. J Investig Med 2002;50:133-39.
35. Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. Br J Clin Pharmacol 2001;52:631-46.
36. Hokanson DE, Sumner DS, Strandness DE, Jr. An electrically calibrated plethysmograph for direct measurement of limb blood flow. IEEE Trans Biomed Eng 1975;22:25-29.
37. Stroes ES, Koomans HA, de Bruin TW, Rabelink TJ. Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. Lancet 1995;346:467-71.
38. Stroes E, Kastelein J, Cosentino F, Erkelens W, Wever R, Koomans H, Luscher T, Rabelink T. Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. J Clin Invest 1997;99:41-46.
39. Chowienczyk PJ, Watts GF, Cockcroft JR, Ritter JM. Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolaemia. Lancet 1992;340:1430-1432.
40. Makimattila S, Liu ML, Vakkilainen J, Schlenzka A, Lahdenpera S, Syvanne M, Mantysaari M, Summanen P, Bergholm R, Taskinen MR, Yki-Jarvinen H. Impaired endothelium-dependent vasodilation in type 2 diabetes. Relation to LDL size, oxidized LDL, and antioxidants. Diabetes Care 1999;22:973-81.
41. Heitzer T, Yla-Herttuala S, Luoma J, Kurz S, Munzel T, Just H, Olschewski M, Drexler H. Cigarette smoking potentiates endothelial dysfunction of forearm resistance vessels in patients with hypercholesterolemia. Role of oxidized LDL. Circulation 1996;93:1346-53.
42. Monnink SH, van Haelst PL, van Boven AJ, Stroes ES, Tio RA, Plokker TW, Smit AJ, Veeger NJ, Crijns HJ, van Gilst WH. Endothelial dysfunction in patients with coronary artery disease: a comparison of three frequently reported tests. J Investig Med 2002;50:19-24.
43. Gordon JB, Ganz P, Nabel EG, Fish RD, Zebede J, Mudge GH, Alexander RW, Selwyn AP. Atherosclerosis influences the vasomotor response of epicardial coronary arteries to exercise. J Clin Invest 1989;83:1946-52.
44. Nabel EG, Ganz P, Gordon JB, Alexander RW, Selwyn AP. Dilation of normal and constriction of atherosclerotic coronary arteries caused by the cold pressor test. Circulation 1988;77:43-52.
45. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111-15.
46. Raitakari OT, Celermajer DS. Flow-mediated dilatation. Br J Clin Pharmacol 2000;50:397-404.
47. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC, . Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995;26:1235-41.
48. Bottcher M, Botker HE, Sonne H, Nielsen TT, Czernin J. Endothelium-dependent and -independent perfusion reserve and the effect of L-arginine on myocardial perfusion in patients with syndrome X. Circulation 1999;99:1795-801.
49. Iliceto S, Marangelli V, Memmola C, Rizzon P. Transesophageal Doppler echocardiography evaluation of coronary blood flow velocity in baseline conditions and during dipyridamole-induced coronary vasodilation. Circulation 1991;83:61-69.

50. Hozumi T, Yoshida K, Akasaka T, Asami Y, Ogata Y, Takagi T, Kaji S, Kawamoto T, Ueda Y, Morioka S. Noninvasive assessment of coronary flow velocity and coronary flow velocity reserve in the left anterior descending coronary artery by Doppler echocardiography: comparison with invasive technique. J Am Coll Cardiol 1998;32:1251-59.
51. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000;101:948-54.
52. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000;101:1899-906.
53. Halcox JPJ, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawin MA, Nour KRA, Quyyumi AA. Prognostic value of coronary vascular endothelial dysfunction. Circulation 2002;106:653-58.
54. Targonski PV, Bonneti PO, Pumper GM, Higano ST, Holmes DR, Lerman A. coronary endothelial dysfunction is associated with an increased risk of cerebrovascular events. Circulation 2003;107:2805-9.
55. von mering GO, Arant CB, Wessel TR, McGorray SP, Merz CN, Sharaf BL, Smith KM, Olson MB, Johnson BD, Sopko G, Handberg EM, Pepine CJ, Kerensky RA. Abnormal Coronary Vasomotion as a Prognostic Indicator of Cardiovascular Events in Women: Results From the National Heart, Lung, and Blood Institute–Sponsored Women's Ischemia Syndrome Evaluation (WISE). Circulation 2004;109:722-25.
56. Asselbergs, F. W., Monnink, S. H., Jessurun, G. A., van Boven, A. J., Veeger, N. J., Zijlstra, F., van Gilst, W. H., and Tio, R. A. Assessing the prognostic value of coronary endothelial function in patients referred for a first coronary angiogram. Am.J Cardiol. 2004.
57. Schindler TH, Hornig B, Buser PT, Olschewski M, Magosaki N, Pfisterer M, Nitzsche EU, Solzbach U, Just H. Prognostic value of abnormal vasoreactivity of epicardial coronary arteries to sympathetic stimulation in patients with normal coronary angiograms. Arterioscler Thromb Vasc Biol 2003;23:495-501.
58. Nitenberg A, Valensi P, Sachs R, Cosson E, Attali JR, Antony I. Prognostic value of epicardial coronary artery constriction to the cold pressor test in type 2 diabetic patients with angiographically normal coronary arteries and no other major coronary risk factors. Diabetes Care 2004;27:208-15.
59. Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, Ferraro A, Chello M, Mastroroberto P, Verdecchia P, Schillaci G. Prognostic significance of endothelial dysfunction in hypertensive patients. Circulation 2001;104:191-96.
60. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673-78.
61. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Rand WM, Udelson JE, Karas RH. Peripheral vascular endothelial function testing as a noninvasive indicator of coronary artery disease. J Am Coll Cardiol 2001;38:1843-49.
62. Anderson TJ, Meredith IT, Yeung AC, Frei B, Selwyn AP, Ganz P. The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. N Engl J Med 1995;332:488-93.
63. Takase B, Uehata A, Akima T, Nagai T, Nishioka T, Hamabe A, Satomura K, Ohsuzu F, Kurita A. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. Am J Cardiol 1998;82:1535-38.
64. Eskurza I, Seals DR, DeSouza CA, Tanaka H. Pharmacologic versus flow-mediated assessments of peripheral vascular endothelial vasodilatory function in humans. Am J Cardiol 2001;88:1067-69.
65. Hijmering ML, Stroes ES, Pasterkamp G, Sierevogel M, Banga JD, Rabelink TJ. Variability of flow mediated dilation: consequences for clinical application. Atherosclerosis 2001;157:369-73.
66. Neunteufl T, Katzenschlager R, Hassan A, Klaar U, Schwarzacher S, Glogar D, Bauer P, Weidinger F. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. Atherosclerosis 1997;129:111-18.
67. Neunteufl T, Heher S, Katzenschlager R, Wolfl G, Kostner K, Maurer G, Weidinger F. Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. Am J Cardiol 2000;86:207-10.
68. Gokce N, Keaney JF, Jr., Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, Vita JA. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. J Am Coll Cardiol 2003;41:1769-75.
69. Gokce N, Keaney JF, Jr., Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. Circulation 2002;105:1567-72.
70. Amoroso G, Tio RA, Mariani MA, van Boven AJ, Jessurun GA, Monnink SH, Grandjean JG, Boonstra PW, Crijns HJ. Functional integrity and aging of the left internal thoracic artery after coronary artery bypass surgery. J Thorac Cardiovasc Surg 2000;120:313-18.
71. Fathi R, Haluska B, Isbel N, Short L, Marwick TH. The relative importance of vascular structure and function in predicting cardiovascular events. J Am Coll Cardiol 2004;43:616-23.
72. Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S, Schoene N, Schuler G. Effect of exercise on coronary endothelial function in patients with coronary artery disease. N Engl J Med 2000;342:454-60.
73. Husain S, Andrews NP, Mulcahy D, Panza JA, Quyyumi AA. Aspirin improves endothelial dysfunction in atherosclerosis. Circulation 1998;97:716-20.
74. Noon JP, Walker BR, Hand MF, Webb DJ. Impairment of forearm vasodilatation to acetylcholine in hypercholesterolemia is reversed by aspirin. Cardiovasc Res 1998;38:480-484.
75. Quyyumi AA. Effects of aspirin on endothelial dysfunction in atherosclerosis. Am J Cardiol 1998;82:31S-3S.
76. Levine GN, Frei B, Koulouris SN, Gerhard MD, Keaney JF, Jr., Vita JA. Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. Circulation 1996;93:1107-13.
77. Gokce N, Keaney JF, Jr., Frei B, Holbrook M, Olesiak M, Zachariah BJ, Leeuwenburgh C, Heinecke JW, Vita JA. Long-term ascorbic acid administration reverses endothelial vasomotor dysfunction in patients with coronary artery disease. Circulation 1999;99:3234-40.
78. Andrews NP, Prasad A, Quyyumi AA. N-acetylcysteine improves coronary and peripheral vascular function. J Am Coll Cardiol 2001;37:117-23.

79. Lieberman EH, Gerhard MD, Uehata A, Walsh BW, Selwyn AP, Ganz P, Yeung AC, Creager MA. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. Ann Intern Med 1994;121:936-41.
80. Gerhard M, Walsh BW, Tawakol A, Haley EA, Creager SJ, Seely EW, Ganz P, Creager MA. Estradiol therapy combined with progesterone and endothelium-dependent vasodilation in postmenopausal women. Circulation 1998;98:1158-63.
81. Saitta A, Altavilla D, Cucinotta D, Morabito N, Frisina N, Corrado F, D'Anna R, Lasco A, Squadrito G, Gaudio A, Cancellieri F, Arcoraci V, Squadrito F. Randomized, double-blind, placebo-controlled study on effects of raloxifene and hormone replacement therapy on plasma no concentrations, endothelin-1 levels, and endothelium-dependent vasodilation in postmenopausal women. Arterioscler Thromb Vasc Biol 2001;21:1512-19.
82. Lind L, Granstam SO, Millgard J. Endothelium-dependent vasodilation in hypertension: a review. Blood Press 2000;9:4-15.
83. Vanhoutte PM. Endothelial dysfunction in hypertension. J Hypertens Suppl 1996;14:S83-S93.
84. Linder L, Kiowski W, Buhler FR, Luscher TF. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo. Blunted response in essential hypertension. Circulation 1990;81:1762-67.
85. Panza JA, Quyyumi AA, Brush JE, Jr., Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 1990;323:22-27.
86. Treasure CB, Manoukian SV, Klein JL, Vita JA, Nabel EG, Renwick GH, Selwyn AP, Alexander RW, Ganz P. Epicardial coronary artery responses to acetylcholine are impaired in hypertensive patients. Circ Res 1992;71:776-81.
87. Brush JE, Jr., Faxon DP, Salmon S, Jacobs AK, Ryan TJ. Abnormal endothelium-dependent coronary vasomotion in hypertensive patients. J Am Coll Cardiol 1992;19:809-15.
88. Panza JA, Garcia CE, Kilcoyne CM, Quyyumi AA, Cannon RO, III. Impaired endothelium-dependent vasodilation in patients with essential hypertension. Evidence that nitric oxide abnormality is not localized to a single signal transduction pathway. Circulation 1995;91:1732-38.
89. Iiyama K, Nagano M, Yo Y, Nagano N, Kamide K, Higaki J, Mikami H, Ogihara T. Impaired endothelial function with essential hypertension assessed by ultrasonography. Am Heart J 1996;132:779-82.
90. Li J, Zhao SP, Li XP, Zhuo QC, Gao M, Lu SK. Non-invasive detection of endothelial dysfunction in patients with essential hypertension. Int J Cardiol 1997;61:165-69.
91. Clozel M, Kuhn H, Hefti F. Effects of angiotensin converting enzyme inhibitors and of hydralazine on endothelial function in hypertensive rats. Hypertension 1990;16:532-40.
92. Novosel D, Lang MG, Noll G, Luscher TF. Endothelial dysfunction in aorta of the spontaneously hypertensive, stroke-prone rat: effects of therapy with verapamil and trandolapril alone and in combination. J Cardiovasc Pharmacol 1994;24:979-85.
93. Rodrigo E, Maeso R, Munoz-Garcia R, Navarro-Cid J, Ruilope LM, Cachofeiro V, Lahera V. Endothelial dysfunction in spontaneously hypertensive rats: consequences of chronic treatment with losartan or captopril. J Hypertens 1997;15:613-18.
94. Hayakawa H, Coffee K, Raij L. Endothelial dysfunction and cardiorenal injury in experimental salt-sensitive hypertension: effects of antihypertensive therapy. Circulation 1997;96:2407-13.
95. Vanhoutte PM, Boulanger CM, Illiano SC, Nagao T, Vidal M, Mombouli JV. Endothelium-dependent effects of converting-enzyme inhibitors. J Cardiovasc Pharmacol 1993;22 Suppl 5:S10-S16.
96. Finta KM, Fischer MJ, Lee L, Gordon D, Pitt B, Webb RC. Ramipril prevents impaired endothelium-dependent relaxation in arteries from rabbits fed an atherogenic diet. Atherosclerosis 1993;100:149-56.
97. Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. J Am Coll Cardiol 2002;40:505-10.
98. Gainer JV, Morrow JD, Loveland A, King DJ, Brown NJ. Effect of bradykinin-receptor blockade on the response to angiotensin-converting-enzyme inhibitor in normotensive and hypertensive subjects. N Engl J Med 1998;339:1285-92.
99. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest 1996;97:1916-23.
100. Antony I, Lerebours G, Nitenberg A. Angiotensin-converting enzyme inhibition restores flow-dependent and cold pressor test-induced dilations in coronary arteries of hypertensive patients. Circulation 1996;94:3115-22.
101. Prasad A, Tupas-Habib T, Schenke WH, Mincemoyer R, Panza JA, Waclawin MA, Ellahham S, Quyyumi AA. Acute and chronic angiotensin-1 receptor antagonism reverses endothelial dysfunction in atherosclerosis. Circulation 2000;101:2349-54.
102. Cheetham C, Collis J, O'Driscoll G, Stanton K, Taylor R, Green D. Losartan, an angiotensin type 1 receptor antagonist, improves endothelial function in non-insulin-dependent diabetes. J Am Coll Cardiol 2000;36:1461-66.
103. Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). J Am Coll Cardiol 2000;35:60-66.
104. Benacerraf S, Carville C, Adnot S, Montagne O, Sediame S, Belhassen L, Dubois-Rande JL. Improvement of bradykinin endothelium-mediated vasodilation of forearm resistance circulation by quinaprilat in patients with coronary artery disease with or without left ventricular dysfunction. J Cardiovasc Pharmacol 1999;34:368-73.
105. Nagy L, Tarjan J, Samoczi M, Kovacs I, Takacs J. Effect of benazepril on endothelial function in previously untreated hypertensive patients.The Working Group of Cardiology of the Academic Committee of Veszprem, Hungary. Am J Ther 1998;5:233-36.
106. Bae JH, Bassenge E, Lee HJ, Park KR, Park CG, Park KY, Lee MS, Schwemmer M. Impact of postprandial hypertriglyceridemia on vascular responses in patients with coronary artery disease: effects of ACE inhibitors and fibrates. Atherosclerosis 2001;158:165-71.

107. Taddei S, Virdis A, Ghiadoni L, Mattei P, Salvetti A. Effects of angiotensin converting enzyme inhibition on endothelium- dependent vasodilatation in essential hypertensive patients. J Hypertens 1998;16:447-56.
108. Lee AF, Dick JB, Bonnar CE, Struthers AD. Lisinopril improves arterial function in hyperlipidaemia. Clin Sci (Lond) 1999;96:441-48.
109. Butler R, Morris AD, Struthers AD. Lisinopril improves endothelial function in chronic cigarette smokers. Clin Sci (Lond) 2001;101:53-58.
110. Bots ML, Remme WJ, Luscher TF, Grobbee DE. PERindopril-Function of the Endothelium in Coronary Artery Disease Trial: the PERFECT study--sub study of EUROPA: rationale and design. Cardiovasc Drugs Ther 2002;16:227-36.
111. Creager MA, Cooke JP, Mendelsohn ME, Gallagher SJ, Coleman SM, Loscalzo J, Dzau VJ. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. J Clin Invest 1990;86:228-34.
112. Drexler H, Zeiher AM. Endothelial function in human coronary arteries in vivo. Focus on hypercholesterolemia. Hypertension 1991;18:II90-II99.
113. Chowienczyk PJ, Watts GF, Cockcroft JR, Ritter JM. Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolaemia. Lancet 1992;340:1430-1432.
114. Casino PR, Kilcoyne CM, Quyyumi AA, Hoeg JM, Panza JA. The role of nitric oxide in endothelium-dependent vasodilation of hypercholesterolemic patients. Circulation 1993;88:2541-47.
115. Seiler C, Hess OM, Buechi M, Suter TM, Krayenbuehl HP. Influence of serum cholesterol and other coronary risk factors on vasomotion of angiographically normal coronary arteries. Circulation 1993;88:2139-48.
116. Shiode N, Kato M, Hiraoka A, Yamagata T, Matsuura H, Kajiyama G. Impaired endothelium-dependent vasodilation of coronary resistance vessels in hypercholesterolemic patients. Intern Med 1996;35:89-93.
117. Voors AA, Oosterga M, Buikema H, May JF, Grandjean JG, van Buiten A, van Gilst WH. Dyslipidemia and endothelium-dependent relaxation in internal mammary arteries used for coronary bypass surgery. Cardiovasc Res 1997;34:568-74.
118. Liao JK, Shin WS, Lee WY, Clark SL. Oxidized low-density lipoprotein decreases the expression of endothelial nitric oxide synthase. J Biol Chem 1995;270:319-24.
119. Liao JK. Inhibition of Gi proteins by low density lipoprotein attenuates bradykinin-stimulated release of endothelial-derived nitric oxide. J Biol Chem 1994;269:12987-92.
120. Galle J, Mulsch A, Busse R, Bassenge E. Effects of native and oxidized low density lipoproteins on formation and inactivation of endothelium-derived relaxing factor. Arterioscler Thromb 1991;11:198-203.
121. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 1993;91:2546-51.
122. Mellwig KP, Baller D, Gleichmann U, Moll D, Betker S, Weise R, Notohamiprodjo G. Improvement of coronary vasodilatation capacity through single LDL apheresis. Atherosclerosis 1998;139:173-78.
123. Igarashi K, Tsuji M, Nishimura M, Horimoto M. Improvement of endothelium-dependent coronary vasodilation after a single LDL apheresis in patients with hypercholesterolemia. J Clin Apheresis 2004;19:11-16.
124. Tamai O, Matsuoka H, Itabe H, Wada Y, Kohno K, Imaizumi T. Single LDL apheresis improves endothelium-dependent vasodilatation in hypercholesterolemic humans. Circulation 1997;95:76-82.
125. Leung WH, Lau CP, Wong CK. Beneficial effect of cholesterol-lowering therapy on coronary endothelium-dependent relaxation in hypercholesterolaemic patients. Lancet 1993;341:1496-500.
126. Avogaro A, Miola M, Favaro A, Gottardo L, Pacini G, Manzato E, Zambon S, Sacerdoti D, de Kreutzenberg S, Piliego T, Tiengo A, Del Prato S. Gemfibrozil improves insulin sensitivity and flow-mediated vasodilatation in type 2 diabetic patients. Eur J Clin Invest 2001;31:603-9.
127. Andrews TC, Whitney EJ, Green G, Kalenian R, Personius BE. Effect of gemfibrozil +/- niacin +/- cholestyramine on endothelial function in patients with serum low-density lipoprotein cholesterol levels <160 mg/dl and high-density lipoprotein cholesterol levels <40 mg/dl. Am J Cardiol 1997;80:831-35.
128. Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors. Arterioscler Thromb Vasc Biol 2001;21:1712-19.
129. Liao JK. Beyond lipid lowering: the role of statins in vascular protection. Int J Cardiol 2002;86:5-18.
130. Wolfrum S, Jensen KS, Liao JK. Endothelium-dependent effects of statins. Arterioscler Thromb Vasc Biol 2003;23:729-36.
131. Laufs U. Beyond lipid-lowering: effects of statins on endothelial nitric oxide. Eur J Clin Pharmacol 2003;58:719-31.
132. Di Napoli P, Antonio TA, Grilli A, Spina R, Felaco M, Barsotti A, De Caterina R. Simvastatin reduces reperfusion injury by modulating nitric oxide synthase expression: an ex vivo study in isolated working rat hearts. Cardiovasc Res 2001;51:283-93.
133. Laufs U, Gertz K, Huang P, Nickenig G, Bohm M, Dirnagl U, Endres M. Atorvastatin upregulates type III nitric oxide synthase in thrombocytes, decreases platelet activation, and protects from cerebral ischemia in normocholesterolemic mice. Stroke 2000;31:2442-49.
134. Bell RM, Yellon DM. Atorvastatin, administered at the onset of reperfusion, and independent of lipid lowering, protects the myocardium by up-regulating a pro-survival pathway. J Am Coll Cardiol 2003;41:508-15.
135. Wolfrum S, Jensen KS, Liao JK. Endothelium-dependent effects of statins. Arterioscler Thromb Vasc Biol 2003;23:729-36.
136. Laufs U, Wassmann S, Hilgers S, Ribaudo N, Bohm M, Nickenig G. Rapid effects on vascular function after initiation and withdrawal of atorvastatin in healthy, normocholesterolemic men. Am J Cardiol 2001;88:1306-7.
137. Omori H, Nagashima H, Tsurumi Y, Takagi A, Ishizuka N, Hagiwara N, Kawana M, Kasanuki H. Direct in vivo evidence of a vascular statin: a single dose of cerivastatin rapidly increases vascular endothelial responsiveness in healthy normocholesterolaemic subjects. Br J Clin Pharmacol 2002;54:395-99.
138. Masumoto A, Hirooka Y, Hironaga K, Eshima K, Setoguchi S, Egashira K, Takeshita A. Effect of pravastatin on endothelial function in patients with coronary artery disease (cholesterol-independent effect of pravastatin). Am J Cardiol 2001;88:1291-94.

139. Mercuro G, Zoncu S, Saiu F, Sarais C, Rosano GM. Effect of atorvastatin on endothelium-dependent vasodilation in postmenopausal women with average serum cholesterol levels. Am J Cardiol 2002;90:747-50.
140. Beckman JA, Liao JK, Hurley S, Garrett LA, Chui D, Mitra D, Creager MA. Atorvastatin restores endothelial function in normocholesterolemic smokers independent of changes in low-density lipoprotein. Circ Res 2004;95:217-23.
141. Sheu WH, Juang BL, Chen YT, Lee WJ. Endothelial dysfunction is not reversed by simvastatin treatment in type 2 diabetic patients with hypercholesterolemia. Diabetes Care 1999;22:1224-25.
142. Sheu WH, Chen YT, Lee WJ. Improvement in endothelial dysfunction with LDL cholesterol level < 80 mg/dl in type 2 diabetic patients. Diabetes Care 2001;24:1499-501.
143. van de Ree MA, Huisman MV, de Man FH, van der Vijver JC, Meinders AE, Blauw GJ. Impaired endothelium-dependent vasodilation in type 2 diabetes mellitus and the lack of effect of simvastatin. Cardiovasc Res 2001;52:299-305.
144. Duffy SJ, O'Brien RC, New G, Harper RW, Meredith IT. Effect of anti-oxidant treatment and cholesterol lowering on resting arterial tone, metabolic vasodilation and endothelial function in the human forearm: a randomized, placebo-controlled study. Clin Exp Pharmacol Physiol 2001;28:409-18.
145. van Etten RW, de Koning EJ, Honing ML, Stroes ES, Gaillard CA, Rabelink TJ. Intensive lipid lowering by statin therapy does not improve vasoreactivity in patients with type 2 diabetes. Arterioscler Thromb Vasc Biol 2002;22:799-804.
146. Economides PA, Caselli A, Tiani E, Khaodhiar L, Horton ES, Veves A. The effects of atorvastatin on endothelial function in diabetic patients and subjects at risk for type 2 diabetes. J Clin Endocrinol Metab 2004;89:740-747.
147. van Venrooij FV, van de Ree MA, Bots ML, Stolk RP, Huisman MV, Banga JD. Aggressive lipid lowering does not improve endothelial function in type 2 diabetes: the Diabetes Atorvastatin Lipid Intervention (DALI) Study: a randomized, double-blind, placebo-controlled trial. Diabetes Care 2002;25:1211-16.
148. Vogel RA, Corretti MC, Plotnick GD. Changes in flow-mediated brachial artery vasoactivity with lowering of desirable cholesterol levels in healthy middle-aged men. Am J Cardiol 1996;77:37-40.
149. Toikka JO, Ahotupa M, Viikari JS, Niinikoski H, Taskinen M, Irjala K, Hartiala JJ, Raitakari OT. Constantly low HDL-cholesterol concentration relates to endothelial dysfunction and increased in vivo LDL-oxidation in healthy young men. Atherosclerosis 1999;147:133-38.
150. van Venrooij FV, van de Ree MA, Bots ML, Stolk RP, Huisman MV, Banga JD. Aggressive lipid lowering does not improve endothelial function in type 2 diabetes: the Diabetes Atorvastatin Lipid Intervention (DALI) Study: a randomized, double-blind, placebo-controlled trial. Diabetes Care 2002;25:1211-16.
151. Cosentino F, Luscher TF. Endothelial dysfunction in diabetes mellitus. J Cardiovasc Pharmacol 1998;32 Suppl 3:S54-S61.
152. Tsunekawa T, Hayashi T, Kano H, Sumi D, Matsui-Hirai H, Thakur NK, Egashira K, Iguchi A. Cerivastatin, a hydroxymethylglutaryl coenzyme a reductase inhibitor, improves endothelial function in elderly diabetic patients within 3 days. Circulation 2001;104:376-79.
153. Vita JA, Yeung AC, Winniford M, Hodgson JM, Treasure CB, Klein JL, Werns S, Kern M, Plotkin D, Shih WJ, Mitchel Y, Ganz P. Effect of cholesterol-lowering therapy on coronary endothelial vasomotor function in patients with coronary artery disease. Circulation 2000;102:846-51.
154. Effect of nifedipine and cerivastatin on coronary endothelial function in patients with coronary artery disease: the ENCORE I Study (Evaluation of Nifedipine and Cerivastatin On Recovery of coronary Endothelial function). Circulation 2003;107:422-28.
155. Noon JP, Walker BR, Hand MF, Webb DJ. Impairment of forearm vasodilatation to acetylcholine in hypercholesterolemia is reversed by aspirin. Cardiovasc Res 1998;38:480-484.
156. Levine GN, Frei B, Koulouris SN, Gerhard MD, Keaney JF, Jr., Vita JA. Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. Circulation 1996;93:1107-13.
157. Gokce N, Keaney JF, Jr., Frei B, Holbrook M, Olesiak M, Zachariah BJ, Leeuwenburgh C, Heinecke JW, Vita JA. Long-term ascorbic acid administration reverses endothelial vasomotor dysfunction in patients with coronary artery disease. Circulation 1999;99:3234-40.
158. Title LM, Cummings PM, Giddens K, Genest JJ, Jr., Nassar BA. Effect of folic acid and antioxidant vitamins on endothelial dysfunction in patients with coronary artery disease. J Am Coll Cardiol 2000;36:758-65.
159. Saitta A, Altavilla D, Cucinotta D, Morabito N, Frisina N, Corrado F, D'Anna R, Lasco A, Squadrito G, Gaudio A, Cancellieri F, Arcoraci V, Squadrito F. Randomized, double-blind, placebo-controlled study on effects of raloxifene and hormone replacement therapy on plasma no concentrations, endothelin-1 levels, and endothelium-dependent vasodilation in postmenopausal women. Arterioscler Thromb Vasc Biol 2001;21:1512-19.
160. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:23-33.
161. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 2000;342:154-60.
162. Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. Postmenopausal hormone replacement therapy: scientific review. JAMA 2002;288:872-81.
163. Liem A, Reynierse-Buitenwerf GH, Zwinderman AH, Jukema JW, van Veldhuisen DJ. Secondary prevention with folic acid: effects on clinical outcomes. J Am Coll Cardiol 2003;41:2105-13.
164. Lange H, Suryapranata H, De Luca G, Borner C, Dille J, Kallmayer K, Pasalary MN, Scherer E, Dambrink JH. Folate therapy and in-stent restenosis after coronary stenting. N Engl J Med 2004;350:2673-81.
165. Hollenberg SM, Klein LW, Parrillo JE, Scherer M, Burns D, Tamburro P, Oberoi M, Johnson MR, Costanzo MR. Coronary endothelial dysfunction after heart transplantation predicts allograft vasculopathy and cardiac death. Circulation 2001;104:3091-96.

166. Asselbergs, F. W., Monnink, S. H., Jessurun, G. A., van Boven, A. J., Veeger, N. J., Zijlstra, F., van Gilst, W. H., and Tio, R. A. Assessing the prognostic value of coronary endothelial function in patients referred for a first coronary angiogram. Am.J Cardiol. 2004.
167. Chan SY, Mancini GB, Kuramoto L, Schulzer M, Frohlich J, Ignaszewski A. The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. J Am Coll Cardiol 2003;42:1037-43.
168. Brevetti G, Silvestro A, Schiano V, Chiariello M. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to ankle-brachial pressure index. Circulation 2003;108:2093-98.
169. Fathi R, Haluska B, Isbel N, Short L, Marwick TH. The relative importance of vascular structure and function in predicting cardiovascular events. J Am Coll Cardiol 2004;43:616-23.
170. Mancini GB, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, Wargovich TJ, Mudra H, Luscher TF, Klibaner MI, Haber HE, Uprichard AC, Pepine CJ, Pitt B. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing ENdothelial Dysfunction) Study. Circulation 1996;94:258-65.
171. Prasad A, Narayanan S, Husain S, Padder F, Waclawiw M, Epstein N, Quyyumi AA. Insertion-deletion polymorphism of the ACE gene modulates reversibility of endothelial dysfunction with ACE inhibition. Circulation 2000;102:35-41.
172. Hirooka Y, Imaizumi T, Masaki H, Ando S, Harada S, Momohara M, Takeshita A. Captopril improves impaired endothelium-dependent vasodilation in hypertensive patients. Hypertension 1992;20:175-80.
173. Kiowski W, Linder L, Nuesch R, Martina B. Effects of angiotensin converting enzyme inhibition on endothelial vasodilator function in primary human hypertension. Eur Heart J 1993;14 Suppl C:5-9.
174. Creager MA, Roddy MA. Effect of captopril and enalapril on endothelial function in hypertensive patients. Hypertension 1994;24:499-505.
175. Iwatsubo H, Nagano M, Sakai T, Kumamoto K, Morita R, Higaki J, Ogihara T, Hata T. Converting enzyme inhibitor improves forearm reactive hyperemia in essential hypertension. Hypertension 1997;29:286-90.
176. O'Driscoll G, Green D, Rankin J, Stanton K, Taylor R. Improvement in endothelial function by angiotensin converting enzyme inhibition in insulin-dependent diabetes mellitus. J Clin Invest 1997;100:678-84.
177. Haefeli WE, Linder L, Luscher TF. Quinaprilat induces arterial vasodilation mediated by nitric oxide in humans. Hypertension 1997;30:912-17.
178. Millgard J, Hagg A, Sarabi M, Lind L. Captopril, but not nifedipine, improves endothelium-dependent vasodilation in hypertensive patients. J Hum Hypertens 1998;12:511-16.
179. O'Driscoll G, Green D, Maiorana A, Stanton K, Colreavy F, Taylor R. Improvement in endothelial function by angiotensin-converting enzyme inhibition in non-insulin-dependent diabetes mellitus. J Am Coll Cardiol 1999;33:1506-11.
180. Houben AJ, Kroon AA, de Haan CH, Fuss-Lejeune MJ, de Leeuw PW. Quinaprilat-induced vasodilatation in forearm vasculature of patients with essential hypertension: comparison with enalaprilat. Cardiovasc Drugs Ther 2000;14:657-63.
181. Higashi Y, Sasaki S, Nakagawa K, Ueda T, Yoshimizu A, Kurisu S, Matsuura H, Kajiyama G, Oshima T. A comparison of angiotensin-converting enzyme inhibitors, calcium antagonists, beta-blockers and diuretic agents on reactive hyperemia in patients with essential hypertension: a multicenter study. J Am Coll Cardiol 2000;35:284-91.
182. Butler R, Morris AD, Burchell B, Struthers AD. DD angiotensin-converting enzyme gene polymorphism is associated with endothelial dysfunction in normal humans. Hypertension 1999;33:1164-68.
183. Mullen MJ, Clarkson P, Donald AE, Thomson H, Thorne SA, Powe AJ, Furuno T, Bull T, Deanfield JE. Effect of enalapril on endothelial function in young insulin-dependent diabetic patients: a randomized, double-blind study. J Am Coll Cardiol 1998;31:1330-1335.
184. Hornig B, Arakawa N, Haussmann D, Drexler H. Differential effects of quinaprilat and enalaprilat on endothelial function of conduit arteries in patients with chronic heart failure. Circulation 1998;98:2842-48.
185. Wilmink HW, Banga JD, Hijmering M, Erkelens WD, Stroes ES, Rabelink TJ. Effect of angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor antagonism on postprandial endothelial function. J Am Coll Cardiol 1999;34:140-145.
186. McFarlane R, McCredie RJ, Bonney MA, Molyneaux L, Zilkens R, Celermajer DS, Yue DK. Angiotensin converting enzyme inhibition and arterial endothelial function in adults with Type 1 diabetes mellitus. Diabet Med 1999;16:62-66.
187. Arcaro G, Zenere BM, Saggiani F, Zenti MG, Monauni T, Lechi A, Muggeo M, Bonadonna RC. ACE inhibitors improve endothelial function in type 1 diabetic patients with normal arterial pressure and microalbuminuria. Diabetes Care 1999;22:1536-42.
188. Esper RJ, Machado R, Vilarino J, Cacharron JL, Ingino CA, Garcia Guinazu CA, Bereziuk E, Bolano AL, Suarez DH. Endothelium-dependent responses in patients with hypercholesterolemic coronary artery disease under the effects of simvastatin and enalapril, either separately or combined. Am Heart J 2000;140:684-89.
189. Bots ML, Remme WJ, Luscher TF, Grobbee DE. PERindopril-Function of the Endothelium in Coronary Artery Disease Trial: the PERFECT study--sub study of EUROPA: rationale and design. Cardiovasc Drugs Ther 2002;16:227-36.
190. Egashira K, Hirooka Y, Kai H, Sugimachi M, Suzuki S, Inou T, Takeshita A. Reduction in serum cholesterol with pravastatin improves endothelium- dependent coronary vasomotion in patients with hypercholesterolemia. Circulation 1994;89:2519-24.
191. Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, Zhang J, Boccuzzi SJ, Cedarholm JC, Alexander RW. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med 1995;332:481-87.

192. Houghton JL, Pearson TA, Reed RG, Torosoff MT, Henches NL, Kuhner PA, Philbin EF. Cholesterol lowering with pravastatin improves resistance artery endothelial function: report of six subjects with normal coronary arteriograms. Chest 2000;118:756-60.
193. Penny WF, Ben Yehuda O, Kuroe K, Long J, Bond A, Bhargava V, Peterson JF, McDaniel M, Juliano J, Witztum JL, Ross J, Jr., Peterson KL. Improvement of coronary artery endothelial dysfunction with lipid- lowering therapy: heterogeneity of segmental response and correlation with plasma-oxidized low density lipoprotein. J Am Coll Cardiol 2001;37:766-74.
194. Wassmann S, Faul A, Hennen B, Scheller B, Bohm M, Nickenig G. Rapid effect of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition on coronary endothelial function. Circ Res 2003;93:e98-103.
195. O'Driscoll G, Green D, Taylor RR. Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within 1 month. Circulation 1997;95:1126-31.
196. Perticone F, Ceravolo R, Maio R, Cloro C, Candigliota M, Scozzafava A, Mongiardo A, Mastroroberto P, Chello M, Mattioli PL. Effects of atorvastatin and vitamin C on endothelial function of hypercholesterolemic patients. Atherosclerosis 2000;152:511-18.
197. John S, Delles C, Jacobi J, Schlaich MP, Schneider M, Schmitz G, Schmieder RE. Rapid improvement of nitric oxide bioavailability after lipid-lowering therapy with cerivastatin within two weeks. J Am Coll Cardiol 2001;37:1351-58.
198. Wassmann S, Ribaudo N, Faul A, Laufs U, Bohm M, Nickenig G. Effect of atorvastatin 80 mg on endothelial cell function (forearm blood flow) in patients with pretreatment serum low-density lipoprotein cholesterol levels <130 mg/dl. Am J Cardiol 2004;93:84-88.
199. Neunteufl T, Kostner K, Katzenschlager R, Zehetgruber M, Maurer G, Weidinger F. Additional benefit of vitamin E supplementation to simvastatin therapy on vasoreactivity of the brachial artery of hypercholesterolemic men. J Am Coll Cardiol 1998;32:711-16.
200. Simons LA, Sullivan D, Simons J, Celermajer DS. Effects of atorvastatin monotherapy and simvastatin plus cholestyramine on arterial endothelial function in patients with severe primary hypercholesterolaemia. Atherosclerosis 1998;137:197-203.
201. Dupuis J, Tardif JC, Cernacek P, Theroux P. Cholesterol reduction rapidly improves endothelial function after acute coronary syndromes. The RECIFE (reduction of cholesterol in ischemia and function of the endothelium) trial. Circulation 1999;99:3227-33.
202. Jarvisalo MJ, Toikka JO, Vasankari T, Mikkola J, Viikari JS, Hartiala JJ, Raitakari OT. HMG CoA reductase inhibitors are related to improved systemic endothelial function in coronary artery disease. Atherosclerosis 1999;147:237-42.
203. Rashid MN, Touchon RC. Dyslipidemia evaluated by brachial artery vasoactivity: a case study of eight patients. W V Med J 1999;95:175-79.
204. Mullen MJ, Wright D, Donald AE, Thorne S, Thomson H, Deanfield JE. Atorvastatin but not L-arginine improves endothelial function in type I diabetes mellitus: a double-blind study. J Am Coll Cardiol 2000;36:410-416.
205. Malik J, Melenovsky V, Wichterle D, Haas T, Simek J, Ceska R, Hradec J. Both fenofibrate and atorvastatin improve vascular reactivity in combined hyperlipidaemia (fenofibrate versus atorvastatin trial--FAT). Cardiovasc Res 2001;52:290-298.
206. Stein JH, Carlsson CM, Papcke-Benson K, Aeschlimann SE, Bodemer A, Carnes M, McBride PE. The effects of lipid-lowering and antioxidant vitamin therapies on flow- mediated vasodilation of the brachial artery in older adults with hypercholesterolemia. J Am Coll Cardiol 2001;38:1806-13.
207. van Venrooij FV, van de Ree MA, Bots ML, Stolk RP, Huisman MV, Banga JD. Aggressive lipid lowering does not improve endothelial function in type 2 diabetes: the Diabetes Atorvastatin Lipid Intervention (DALI) Study: a randomized, double-blind, placebo-controlled trial. Diabetes Care 2002;25:1211-16.
208. Dogra GK, Watts GF, Herrmann S, Thomas MA, Irish AB. Statin therapy improves brachial artery endothelial function in nephrotic syndrome. Kidney Int 2002;62:550-557.
209. Omori H, Nagashima H, Tsurumi Y, Takagi A, Ishizuka N, Hagiwara N, Kawana M, Kasanuki H. Direct in vivo evidence of a vascular statin: a single dose of cerivastatin rapidly increases vascular endothelial responsiveness in healthy normocholesterolaemic subjects. Br J Clin Pharmacol 2002;54:395-99.
210. de Jongh S, Lilien MR, op't RJ, Stroes ES, Bakker HD, Kastelein JJ. Early statin therapy restores endothelial function in children with familial hypercholesterolemia. J Am Coll Cardiol 2002;40:2117-21.
211. Vita JA, Gokce N, Duffy SJ, Kahn D, Tomasian D, Palmisano J, Thomas S, Holbrook M, Keaney JF, Jr. Effect of atorvastatin on endothelium-dependent vasodilation in patients with coronary artery disease. Am J Cardiol 2003;91:857-60.
212. van Haelst PL, van Doormaal JJ, Asselbergs FW, Van Roon AM, Veeger NJ, Henneman MM, Smit AJ, Cohen Tervaert JW, May JF, Gans RO. Correlates of endothelial function and their relationship with inflammation in patients with familial hypercholesterolaemia. Clin Sci (Lond) 2003;104:627-32.
213. Economides PA, Caselli A, Tiani E, Khaodhiar L, Horton ES, Veves A. The effects of atorvastatin on endothelial function in diabetic patients and subjects at risk for type 2 diabetes. J Clin Endocrinol Metab 2004;89:740-747.
214. Beckman JA, Liao JK, Hurley S, Garrett LA, Chui D, Mitra D, Creager MA. Atorvastatin restores endothelial function in normocholesterolemic smokers independent of changes in low-density lipoprotein. Circ Res 2004;95:217-23.