

Pulse Pressure and Endothelial Dysfunction in Never-Treated Hypertensive Patients

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OBJECTIVES	This study sought to investigate whether pulse pressure (PP) is associated with endothelium-dependent vasodilation in a group of never-treated hypertensives.
BACKGROUND	Pulse pressure represents a well-established independent predictor for cardiovascular morbidity and mortality. Forearm endothelial dysfunction, defined as impaired vasodilating response to acetylcholine (ACh), may be associated with several cardiovascular risk factors. Recently, the prognostic value of coronary and forearm endothelial dysfunction has been demonstrated.
METHODS	All patients underwent measurement of blood pressure (BP) both clinically and in an ambulatory setting. Endothelium-dependent and -independent vasodilation was investigated by strain-gauge plethysmography in 262 hypertensive patients (age 30 to 55 years) during intra-arterial infusion of increasing doses of ACh and sodium nitroprusside.
RESULTS	We observed that systolic BP rather than diastolic BP significantly induces the PP increase. Linear regression analysis revealed a significant inverse correlation between ACh-stimulated forearm blood flow (FBF) and age, body mass index, clinic and monitored systolic BP, and clinic and monitored PP. However, stepwise multivariate analysis showed that monitored PP was the strongest independent predictor of ACh-stimulated FBF, accounting for 33.6% of the variation. After adjustment for other covariates, ACh-stimulated FBF decreases by 8.7% for each mm Hg increment in monitored PP.
CONCLUSIONS	Our data indicate that monitored PP is inversely correlated with ACh-stimulated vasodilation. It is possible to hypothesize that elevation in PP reduces FBF by increasing oxidative stress and reducing production of nitric oxide caused by reduced shear stress. In addition, the present findings demonstrate the accuracy of ambulatory BP as a prognostic predictor of hypertension-associated endothelial dysfunction. (J Am Coll Cardiol 2003;41:1753-8) © 2003 by the American College of Cardiology Foundation

Pulse pressure (PP), defined as the difference between systolic and diastolic blood pressure (BP), represents a well-established independent predictor for cardiovascular morbidity and mortality (1-4). A possible explanation for the association between PP and adverse cardiovascular outcomes may be provided by the concept of bidirectionality: an increased PP is both a cause and a consequence of atherosclerosis (4).

See page 1759

The vascular endothelium plays a pivotal role in the regulation of vascular tone and the maintenance of cardiovascular homeostasis by the release of some vasoactive factors. Receptor-dependent agonists—such as acetylcholine (ACh), bradykinin, and substance P—and mechanical stimuli, such as shear stress, relax vascular wall by the release of nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF). The mechanism of action of EDHF seems to be different from the mechanism of action of the L-arginine-NO pathway because it involves

calcium-sensitive K⁺ channels, which are sensitive to the combined effects of the apamin and charybdotoxin (5), as well as cytochrome P450 metabolites (6).

Dysfunction of the vascular endothelium, due to a decreased bioavailability of NO, plays an important role in the appearance and progression of atherosclerosis (7,8). Recently, it has been reported that both coronary (9,10) and forearm (11) endothelial dysfunction predict long-term atherosclerotic disease progression and cardiovascular events rate.

Recent studies have shown an association between PP elevation and endothelial dysfunction assessed by ACh reactivity in intact carotid arteries from rabbits (12). However, no data are available about the association between PP elevation and ACh-stimulated vasodilation in human hypertension. To these observations, we investigated whether PP elevation is associated with endothelial dysfunction in a group of never-treated hypertensive patients.

METHODS

Study population. A total of 262 outpatients at Catanzaro University Hospital, 130 men and 132 women, age 30 to 55 years (mean \pm SD = 46.1 \pm 5.7), with well-documented history of essential hypertension were included in the study; 225 of them have been included in our previous report (11). Patient characteristics and inclusion criteria were previously

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Abbreviations and Acronyms

ACh	= acetylcholine
ANOVA	= analysis of variance
BMI	= body mass index
BP	= blood pressure
EDHF	= endothelium-derived hyperpolarizing factor
FBF	= forearm blood flow
NO	= nitric oxide
PP	= pulse pressure
SNP	= sodium nitroprusside
VR	= vascular resistance

described (11). All patients were Caucasian and underwent physical examination and review of their medical histories. Causes of secondary hypertension were excluded by clinical and biochemical tests. At the time of vascular evaluation, none of the patients had history or clinical evidence of angina, myocardial infarction, valvular heart disease, diabetes, hyperlipidemia, peripheral vascular disease, coagulopathy, or any disease predisposing them to vasculitis or Raynaud's phenomenon. Body mass index (BMI) ranged from 22 to 28 kg/m². No participant had ever been treated with antihypertensive drugs. The local ethics committee approved the study, and all participants gave written informed consent for all procedures.

BP measurements. Readings of clinic BP were obtained in the left arm of the supine patients, after 5 min of quiet rest, with a mercury sphygmomanometer. A minimum of three BP readings were taken on three separate occasions at least two weeks apart. Systolic and diastolic BP were recorded at the first appearance (phase I) and the disappearance (phase V) of Korotkoff sounds. Baseline BP values were the average of the last two of the three consecutive measurements obtained at intervals of 3 min. Patients with a clinic BP ≥ 140 mm Hg systolic and/or ≥ 90 mm Hg diastolic were defined as hypertensive.

Ambulatory BP monitorings were obtained by using an A&D TM-2420/2421 recorder (A&D, Takeda, Japan). Recordings were taken every 10 min during the day (from 7:00 AM to 11:00 PM) and every 20 min during the night (from 11:00 PM to 7:00 AM).

Forearm blood flow (FBF) measurements. All studies were performed at 9:00 AM after overnight fasting, with the subjects lying supine in a quiet, air-conditioned room (22°C to 24°C). The subjects were instructed to continue their regular diet; caffeine, alcohol, and smoking were all allowed within at least 24 h before the study. Forearm volume was determined by water displacement. Under local anesthesia and sterile conditions, a 20-gauge polyethylene catheter (Vasculon 2, Baxter Healthcare Corp., Deerfield, Illinois) was inserted into the brachial artery of the nondominant arm of each subject for evaluation of BP (Baxter Healthcare Corp.) and for drug infusion. This arm was slightly elevated above the level of the right atrium, and a mercury-filled silastic strain-gauge was placed on the widest part of the

forearm. The strain-gauge was connected to a plethysmograph (model EC-4, D.E. Hokanson, Issaquah, Washington) calibrated to measure the percent change in volume; this was connected to a chart recorder to obtain the FBF measurements. A cuff placed on the upper arm was inflated to 40 mm Hg with a rapid cuff inflator (model E-10, D.E. Hokanson) to exclude venous outflow from the extremity. A wrist cuff was inflated to BP values 1 min before each measurement to exclude the hand blood flow. The antecubital vein of the opposite arm was cannulated.

The FBF was measured as the slope of the change in the forearm volume. The mean of at least three measurements was obtained at each time point. Forearm vascular resistance (VR), as expressed in units, was calculated by dividing mean BP by FBF.

Vascular function. The protocol, previously described by Panza et al. (13) and subsequently used by our group (11,14-16), was employed for the present study. All patients underwent measurement of FBF and BP during intra-arterial infusion of saline, ACh, and sodium nitroprusside (SNP) at increasing doses. All participants rested 30 min after artery cannulation to reach a stable baseline before data collection; measurements of FBF and VR, expressed in units, were repeated every 5 min until stable.

Endothelium-dependent and -independent vasodilation were assessed by a dose-response curve to intra-arterial ACh infusions (7.5, 15, and 30 $\mu\text{g}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$, each for 5 min) and SNP infusions (0.8, 1.6, and 3.2 $\mu\text{g}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$, each for 5 min), respectively. The sequence of administration of ACh and SNP was randomized to avoid any bias related to the order of drug infusion. The drug infusion rate, adjusted for forearm volume of each subject, was 1 ml/min.

Drugs. Acetylcholine (Sigma, Milan, Italy) was diluted with saline immediately before infusion. Sodium nitroprusside (Malesci, Florence, Italy) was diluted in 5% glucose solution immediately before each infusion and protected from light with aluminum foil.

Statistical analysis. Standard descriptive and comparative analyses were undertaken. The vasodilating responses to ACh and SNP were compared by analysis of variance (ANOVA) for repeated measurements and, when analysis was significant, the Tukey test was applied. The effects of independent predictors on peak percent increase on FBF were evaluated by a simple linear regression analysis and, successively, by a stepwise multiple linear regression with forward selection. At first, we tested a baseline model by using the following variables: age, gender, BMI, serum glucose, serum cholesterol, serum triglycerides, and smoking habits (previous or current smokers or never smoked). Subsequent improvements in the model fitting were tested by entering, one at a time, the different clinic and monitored BP components: systolic BP, diastolic BP, mean BP, and PP. Parametric data are reported as mean \pm SD. Significant differences were assumed to be at $p < 0.05$. All comparisons were performed using the statistical package SPSS 10.0 for Windows (SPSS Inc., Chicago, Illinois).

Table 1. Study Population Characteristics

Gender, males/females	130/132
Age, yrs	46.1 ± 5.7
Body mass index, kg/m ²	26.2 ± 1.5
Current smokers, %	24.9
Total cholesterol, mmol/l	4.9 ± 0.6
Triglycerides, mmol/l	1.31 ± 0.2
Fasting glucose, mmol/l	5.05 ± 0.5
Clinic systolic BP, mm Hg	158 ± 13
Clinic diastolic BP, mm Hg	94 ± 8
24-h systolic BP, mm Hg	148 ± 10
24-h diastolic BP, mm Hg	89 ± 7
Clinic PP, mm Hg	64 ± 11
24-h PP, mm Hg	59 ± 9
Baseline FBF, ml·100 tissue ⁻¹ ·min ⁻¹	3.3 ± 0.6
Baseline vascular resistance, U	33.6 ± 5.4

BP = blood pressure; FBF = forearm blood flow; PP = pulse pressure.

RESULTS

Clinical, humoral, and hemodynamic characteristics of the study population are reported in Table 1.

Endothelium-dependent vasodilation. Intra-arterial infusion of ACh caused a significant ($p < 0.0001$) dose-dependent increase in FBF and decrease in forearm VR. The FBF increments from basal measurements at the three incremental doses of ACh were as follows: 2.1 ± 1.3 ml·100 ml⁻¹ of tissue·min⁻¹ (+65%), 5.1 ± 2.7 ml·100 ml⁻¹ of tissue·min⁻¹ (+153%), 10.2 ± 4.9 ml·100 ml⁻¹ of tissue·min⁻¹ (+307%). At the highest dose of ACh (30 μg/min), FBF increased to 13.5 ± 5.2 ml·100 ml⁻¹ of tissue·min⁻¹ and VR decreased to 10.6 ± 4.9 U. Intra-arterial infusion of ACh caused no change in BP or heart rate values.

Endothelium-independent vasodilation. Increasing doses of intra-arterial infusion of SNP induced a significant ($p < 0.0001$) increase in FBF as well as a decrease in forearm VR. The FBF percent increments from basal measurements were $80 \pm 38\%$, $169 \pm 63\%$, and $354 \pm 84\%$; VR decreased from basal measurements to 22.4 ± 6.4 U, 16.5 ± 4.5 U, and 8.3 ± 2.3 U, respectively. Intra-arterial infusion of SNP caused no changes in BP or heart rate values.

Correlational analyses. Initially, we performed a simple linear regression analysis between the peak percent increase in both ACh- and SNP-stimulated FBF and the following variables: age, BMI, serum glucose, serum cholesterol, serum triglycerides, and both clinic and monitored BP components (Table 2). As shown in Table 2, age, BMI, and clinic and monitored systolic BP and PP were significantly correlated with the peak percent increase in ACh-stimulated FBF; on the contrary, no significant correlations were detected using SNP-stimulated FBF as a dependent variable. In particular, the peak percent increase in FBF after intra-arterial ACh infusions was inversely related to clinic systolic BP ($r = -0.372$; $p < 0.0001$) and PP ($r = -0.444$; $p < 0.0001$), monitored systolic BP ($r = -0.474$; $p < 0.0001$), and PP ($r = -0.578$; $p < 0.0001$), accounting for 13.9% and 19.8%, and for 22.5% and 33.5% of the

Table 2. Results of Univariate Linear Regression Analysis Between Different Covariates and Peak in ACh- and SNP-Stimulated Forearm Blood Flow

	ACh		SNP	
	r	P	r	P
Age	-0.133	0.031	0.087	0.160
Total cholesterol	-0.002	0.905	0.080	0.196
Triglycerides	-0.095	0.125	0.065	0.297
Body mass index	-0.233	0.0001	0.029	0.641
Fasting glucose	-0.019	0.763	0.093	0.133
Clinic systolic BP	-0.372	0.001	0.093	0.132
Clinic diastolic BP	-0.024	0.069	0.037	0.548
Clinic PP	-0.444	0.0001	0.090	0.145
24-h systolic BP	-0.476	0.0001	0.112	0.270
24-h diastolic BP	0.148	0.523	0.031	0.615
24-h pulse pressure	-0.579	0.0001	0.102	0.101

ACh = acetylcholine; BP = blood pressure; PP = pulse pressure; SNP = sodium nitroprusside.

variation in ACh-stimulated FBF, respectively. After linear regression we performed a stepwise multivariate analysis to identify the independent predictors of ACh-stimulated FBF. In this way, we observed that the addition of BP components to the baseline model (including age, gender and BMI) that accounts for the 10.5% of the variation, significantly increased the FBF variation to 22.6% ($p < 0.0001$) for the clinic systolic BP, 29.9% ($p < 0.0001$) for the clinic PP, 31.3% ($p < 0.0001$) for the monitored systolic BP, and 42.8% ($p < 0.0001$) for the monitored PP. The addition of monitored systolic BP accounts for another 1.1% of the variation; thus, the total model accounts for 43.9% ($p < 0.0001$) of FBF variation. As shown in Table 3, the monitored PP was an independent and strong predictor of ACh-stimulated FBF, accounting for 33.6% ($p < 0.0001$) of the variation. When monitored PP and systolic BP were entered contemporary into the same model, this latter did not reach statistical significance. Thus, the final model indicates that for each mm Hg increase in monitored PP, the ACh-stimulated FBF decreases by 8.7%.

We also evaluated the relationship between the increase in ACh-stimulated FBF and the distribution of patients into quartiles according to levels of monitored PP: <53 mm Hg (lower quartile), from 53 to 58 mm Hg (second quartile), from 58 to 65 mm Hg (third quartile), and >65 mm Hg (upper quartile). The mean peak percent increases in FBF into quartiles were 430 ± 107 , 326 ± 117 , 251 ± 105 , and 219 ± 91 , respectively ($p < 0.0001$, by ANOVA)

Table 3. Independent Predictors of Peak Increase in ACh-Stimulated Forearm Blood Flow After Multivariate Analysis

	Partial r ² (%)	Total r ² (%)	Significance (P)
24-h PP, mm Hg	33.6	33.6	<0.0001
Gender, male	4.9	38.5	<0.0001
Body mass index, kg/m ²	2.5	41.0	<0.002
Age, yrs	1.8	42.8	<0.005
24-h systolic BP, mm Hg	1.1	43.9	<0.05

ACh = acetylcholine; BP = blood pressure; PP = pulse pressure.

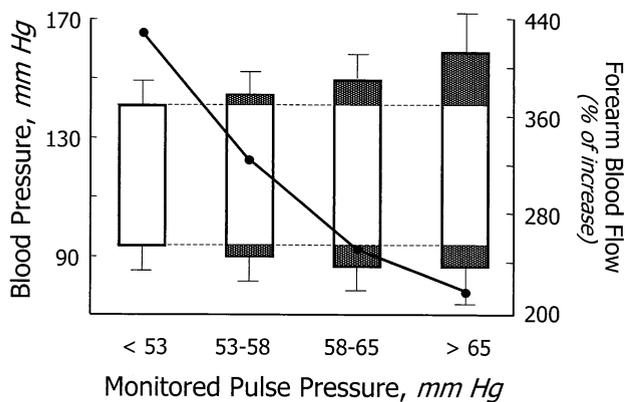


Figure 1. Blood pressure (bars) and peak percent increase in forearm blood flow (line) values by quartiles of pulse pressure are graphically reported. It is evident that forearm blood flow values progressively decrease from lower to upper quartile of pulse pressure. The picture also demonstrates the contribution of both systolic and diastolic blood pressure (dark zone) to increase in pulse pressure. Particularly, from lower to upper quartile of pulse pressure, systolic blood pressure increases by 17 mm Hg, whereas diastolic blood pressure decreases by 6 mm Hg.

(Fig. 1). The major determinant of the PP increase among the quartiles was the systolic BP. In fact, systolic BP increased from 141 ± 6 mm Hg (lower quartile) to 158 ± 10 mm Hg (upper quartile) ($p < 0.0001$ by ANOVA), whereas diastolic BP decreased from 93 ± 6 mm Hg (lower quartile) to 87 ± 9 mm Hg (upper quartile) ($p < 0.0001$ by ANOVA).

DISCUSSION

In this study, we demonstrated that monitored PP is inversely related to ACh-stimulated vasodilation in a large sample of initially untreated and uncomplicated hypertensive patients. This relation persists after adjustment for the significant influence of other covariates. These data are consistent with those previously reported in an experimental animal model demonstrating that PP modulates ACh-induced, endothelium-dependent responses (12). In addition, our data outline the accuracy of ambulatory BP to highlight the association between pressure overload and hypertension-associated endothelial dysfunction. The present data also demonstrate that systolic BP rather than diastolic BP significantly contributes to the increase in PP. In fact, after partitioning PP in quartiles, the diastolic BP decreases, from lower to upper quartile, only by 6 mm Hg, whereas systolic BP increases by 17 mm Hg.

Hypertension and endothelial function. The normal endothelium modulates local blood flow and prevents the appearance and progression of atherosclerosis through the dynamic release of different vasoactive factors. Even if many of the vascular protective actions of endothelium are mainly dependent on NO production, endothelium-dependent vasorelaxation is mediated, at least in part, by other vasoactive substances, such as EDHF. In particular, the EDHF-related vasodilation seems to compensate for the impaired NO-dependent vasodilation in essential hypertension. The

most important endothelium-derived relaxing factor is NO that may be released after stimulation by endogenous and pharmacologic agonists and physical stimuli (17-20), such as flow-mediated shear stress.

On the other hand, it is well known that in forearm vasculature of hypertensive patients endothelium-dependent vasodilation is impaired (11,13-16,19-21), accounting for the increase in VR and for the vascular structural changes. Nevertheless, other studies indicate that endothelial dysfunction may contribute to the pathogenesis of essential hypertension by offsetting the balance between vasodilator and vasoconstrictor forces on vasculature (22,23). This abnormality, probably multifactorial, is mainly related to a decreased NO bioavailability that may follow a reduction of NO synthesis and/or its increased inactivation by oxidative stress. Thus, endothelial dysfunction, which is characterized by reduced endothelium-dependent vasodilation and proinflammatory, proliferative, and procoagulatory properties may be considered the initial modification, present in patients with essential hypertension and other cardiovascular risk factors, that promotes the coronary and extracoronary atherosclerosis (7,8,17-20). In fact, a dysfunctioning endothelium increases vascular tone and platelets and monocytes adhesion, and stimulates the proliferation of vascular smooth muscle cells and fibroblasts. Moreover, endothelium modulates other important processes in the development of atherosclerosis, including inflammation and thrombosis.

Flow-mediated shear stress and endothelium. The normal endothelium contributes to the maintenance of a constant flow-mediated shear stress by releasing vasoactive agents that modulate vascular diameter. This regulatory function is clinically relevant because different levels of shear stress interact with experimental atherogenesis modulating NO production (24-26). Similarly, human studies have shown that low flow-mediated shear stress impairs endothelium-dependent vasodilation both in coronary (27) and peripheral (28) conductance arteries.

Interestingly, flow pulsatility, but not turbulent flow, was observed at the sites of atherosclerotic plaques (29). Thus, on the basis of these observations, it is evident that oscillatory and steady laminar shear stress exert differential effects on endothelial cells. Indeed, De Keulenaer et al. (30) have recently reported that continuous oscillatory shear stress causes a sustained activation of prooxidant processes resulting in redox-sensitive gene expression in human umbilical endothelial cells. The findings by De Keulenaer were extended by Silacci et al. (31), who showed that pulsatile flow increases endothelial oxidative stress similar to oscillatory flow conditions. These findings may be explained by the up-regulation of NO synthase III with a mechanism involving, at least in part, the activation of the nuclear factor-kappa B resulting in an increase of oxidative stress. In keeping with these, laminar flow-mediated shear stress rather than oscillatory shear stress appears to exert protective antiatherosclerotic vascular effects.

BP and flow-mediated shear stress. It is well established that local factors and vascular geometry modulate the blood flow pattern and velocity and, thus, the level of flow-mediated shear stress (32). An increase in vascular diameter may account for the observed inverse relationship between systolic BP and wall shear stress (33). Thus, vascular changes induced by hypertension may affect the magnitude of flow-mediated shear stress and, thereby, NO production.

In arterial BP it is possible to recognize a steady component, as mean BP, and a pulsatile component, as PP. Major determinants of mean BP are considered ventricular ejection and peripheral VR, whereas ventricular ejection and vascular wall stiffness contribute to PP (34). Therefore, it is reasonable to hypothesize that the same experimental mechanisms are operating in the endothelial dysfunction of human hypertension. Consistent with this hypothesis, even if we did not measure the oxidative stress, it is possible to speculate that elevation in PP reduces ACh-stimulated vasodilation by increasing oxidative stress and reducing NO production as consequence of low shear stress. These mechanisms, even if they remain still too speculative, are consistent with previous experimental findings (28–31). On the other hand, Wilkinson and coworkers (35) recently demonstrated that endogenous NO regulates arterial distensibility *in vivo*, whereby an increase in endothelium-derived NO is associated with reduced arterial stiffness. Thus, it is equally plausible that reduced NO bioavailability induces a PP elevation and that increase in PP reduces NO production, supporting the concept that an elevated PP is both a cause and a consequence of atherosclerosis (4).

Clinical implications. Increase in PP has been related to cardiac and vascular hypertrophy (36), coronary heart disease (1,3), and subsequent cardiovascular events, resulting in a good or better predictor than other BP components (1–4). Therefore, consistent with these findings, it might be useful to consider PP an important and new clinical predictor to define the total cardiovascular risk profile of hypertensive patients.

Even if it is premature to identify the reduction of PP as a therapeutic goal, it is reasonable to affirm that cardiovascular risk in hypertensive patients could be further reduced by narrowing the pulsatile component of BP at any reduction in mean BP. Angiotensin-converting enzyme inhibitors, calcium channel blockers, and low-dose diuretics seem to be effective in improving arterial distensibility (4).

Conclusions. The present study demonstrates that PP is a strong independent predictor of endothelium-dependent ACh-stimulated vasodilation in hypertensive patients. This response probably is mediated by magnitude and type of shear stress. Finally, our findings extend previous observations about possible mechanisms operating in endothelial function.

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REFERENCES

1. Franklin SS, Khan SA, Wong ND, Larson MG, Levy D. Is pulse pressure useful in predicting risk for coronary heart disease? The Framingham heart study. *Circulation* 1999;100:354–60.
2. Millar JA, Lever AF. Implications of pulse pressure as a predictor of cardiac risk in patients with hypertension. *Hypertension* 2000;36:907–11.
3. Verdecchia P, Schillaci G, Reboldi G, Franklin SS, Porcellati C. Different prognostic impact of 24-hour mean blood pressure on stroke and coronary artery disease in essential hypertension. *Circulation* 2001;103:2579–84.
4. Dart AM, Kingwell BA. Pulse pressure. A review of mechanisms and clinical relevance. *J Am Coll Cardiol* 2001;37:975–84.
5. Edwards G, Dora KA, Gardener MJ, et al. K⁺ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature* 1998;396:269–72.
6. Coats P, Johnston F, MacDonald J, McMurray JJ, Hillier C. Endothelium-derived hyperpolarizing factor. Identification and mechanisms of action in human subcutaneous resistance arteries. *Circulation* 2001;103:1702–8.
7. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease. *N Engl J Med* 1992;326:242–50.
8. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1998;334:1349–57.
9. 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.
10. Schächinger 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.
11. Perticone F, Ceravolo R, Pujia A, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 2001;104:191–6.
12. Ryan SM, Waack BJ, Weno BL, Heistad DD. Increases in pulse pressure impair acetylcholine-induced vascular relaxation. *Am J Physiol* 1995;268:H359–63.
13. Panza JA, Quyyumi AA, Brush JE, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990;323:22–7.
14. Perticone F, Ceravolo R, Maio R, et al. Angiotensin-converting enzyme gene polymorphism is associated with endothelium-dependent vasodilation in never treated hypertensive patients. *Hypertension* 1998;31:900–5.
15. Perticone F, Maio R, Ceravolo R, Cosco C, Cloro C, Mattioli PL. Relationship between left ventricular mass and endothelium-dependent vasodilation in never-treated hypertensive patients. *Circulation* 1999;99:1991–6.
16. Perticone F, Ceravolo R, Maio R, et al. Calcium antagonist isradipine improves abnormal endothelium-dependent vasodilation in never treated hypertensive patients. *Cardiovasc Res* 1999;41:299–306.
17. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288:373–6.
18. Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. *N Engl J Med* 1990;323:27–36.
19. Lüscher TF, Vanhoutte PM. The Endothelium: Modulator of Cardiovascular Function. Boca Raton, FL: CRC Press, 1990.
20. Quyyumi AA. Endothelial function in health and disease: new insights into the genesis of cardiovascular disease. *Am J Med* 1998;105:32S–9S.
21. Paniagua OA, Bryant MB, Panza JA. Role of endothelial nitric oxide in shear stress-induced vasodilation of human microvasculature. Diminished activity in hypertensive and hypercholesterolemic patients. *Circulation* 2001;103:1752–8.
22. Panza JA, Quyyumi AA, Callahan TS, Epstein SE. Effect of antihypertensive treatment on endothelium-dependent vascular relaxation in patients with essential hypertension. *J Am Coll Cardiol* 1993;21:1145–51.
23. Taddei S, Virdis A, Mattei P, Arzilli F, Salvetti A. Endothelium-dependent forearm vasodilation is reduced in normotensive subjects with familial history of hypertension. *J Cardiovasc Pharmacol* 1992;20:S193–5.

24. Zarins CK, Bomberger RA, Glagov S. Local effects of stenoses: increased flow velocity inhibits atherogenesis. *Circulation* 1981;64 Suppl II:221-7.
25. Koller A, Sun D, Kaley G. Corelease of nitric oxide and prostaglandins mediates flow dependent dilation of gracilis muscle arterioles. *Am J Physiol* 1994;267:H326-32.
26. Ziegler T, Bouzourene K, Harrison VJ, Brunner HR, Hayoz D. Influence of oscillatory and unidirectional flow environments on the expression of endothelin and nitric oxide synthase in cultured endothelial cells. *Arterioscler Thromb Vasc Biol* 1998;18:686-92.
27. Drexler H, Zeiher AM, Wollschlaenger H, Meinertz T, Just H, Bonzel T. Flow-dependent coronary artery dilation in humans. *Circulation* 1989;80:466-74.
28. Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340:1111-5.
29. Ziegler T, Silacci P, Harrison VJ, Hayoz D. Nitric oxide synthase expression in endothelial cells exposed to mechanical forces. *Hypertension* 1998;32:351-5.
30. De Keulenaer GW, Chappell DC, Ishizaka N, Nerem RM, Alexander RW, Griendling KK. Oscillatory and steady laminar shear stress differentially affect human endothelial redox state: role of a superoxide-producing NADH oxidase. *Circ Res* 1998;82:1094-101.
31. Silacci P, Desgeorges A, Mazzolai L, Chambaz C, Hayoz D. Flow pulsatility is a critical determinant of oxidative stress in endothelial cells. *Hypertension* 2001;38:1162-6.
32. Fisher M, Fieman S. Geometric factors of the bifurcation in carotid atherogenesis. *Stroke* 1990;21:267-71.
33. Gnasso A, Carallo C, Irace C, et al. Association between intima-media thickness and wall shear stress in common carotid arteries in healthy male subjects. *Circulation* 1996;94:3257-62.
34. Nichols WW, O'Rourke MF. *McDonald's Blood Flow in Arteries*. Philadelphia, PA: Lea & Febiger, 1988.
35. Wilkinson IB, Qasem A, McEniery CM, Webb DJ, Avolio AP, Cockcroft JR. Nitric oxide regulates local arterial distensibility in vivo. *Circulation* 2002;105:213-7.
36. Khattar RS, Acharya DU, Kinsey C, Senior R, Lahiri A. Longitudinal association of ambulatory pulse pressure with left ventricular mass and vascular hypertrophy in essential hypertension. *J Hypertens* 1997;15:737-43.