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Women and Cardiovascular Disease

Prognostic Role of Reversible Endothelial Dysfunction in Hypertensive Postmenopausal Women

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OBJECTIVES The aim of the present study was to assess whether optimized antihypertensive treatment is effective in modifying endothelial function and whether an improvement in flow-mediated vasodilation (FMD) in response to treatment, as an expression of reversible endothelial dysfunction, could predict a more favorable prognosis in a population of postmenopausal women.

BACKGROUND Hypertensive postmenopausal women have been shown to have abnormal endothelium-dependent vascular function. However, FMD may change over time, according to antihypertensive treatment; the prognostic value of these changes has not been investigated.

METHODS A total of 400 consecutive postmenopausal women with mild-to-moderate hypertension and impaired FMD underwent ultrasonography of the brachial artery at baseline and after six months, while optimal control of blood pressure was achieved using antihypertensive therapy. They were then followed up for a mean period of 67 months (range 57 to 78). Endothelial function was measured as FMD of the brachial artery, using high-resolution ultrasound.

RESULTS After six months of treatment, FMD had not changed (≤10% relative to baseline) in 150 (37.5%) of 400 women (group 1), whereas it had significantly improved (>10% relative to baseline) in the remaining 250 women (62.5%) (group 2). During follow-up, we noticed 32 events (3.50 per 100 person-years) in group 1 and 15 events (0.51 per 100 person-years) in group 2 ($p < 0.0001$).

CONCLUSIONS This study demonstrates that a significant improvement in endothelial function may be obtained after six months of antihypertensive therapy and clearly identifies patients who possibly have a more favorable prognosis. (J Am Coll Cardiol 2002;40:505–10) © 2002 by the American College of Cardiology Foundation

It is now well recognized that the endothelium plays a fundamental role in the regulation of vascular tone by releasing a variety of vasodilator substances—first and foremost, nitric oxide (NO)—that modulate the contractile behavior of the underlying vascular smooth muscle cells (1,2). The most important consequence of normal endothelial function in vivo is the ability to release NO in response to physiologic stimuli, such as increases in flow, reflecting flow-dependent endothelium-mediated dilation (flow-mediated dilation [FMD]) (3–5). Recently, it has been demonstrated that high-resolution ultrasound study of endothelium-dependent brachial artery FMD is a useful tool for assessing the change in vasomotor response relating to drug therapy (6–9). Several studies have confirmed that FMD of the brachial artery is impaired in patients with various atherosclerotic risk factors, including advanced age, hyperlipidemia, hypertension, diabetes mellitus, tobacco use, and postmenopausal status (6,8,10–23). In particular, hypertensive postmenopausal women have been shown to have abnormal endothelial function (24). However, endothelial dysfunction may reverse over time, according to antihypertensive treatment (6,8,22–27). In other words, an optimal therapeutic regimen may normalize FMD; but, to date, this phenomenon has not been demonstrated in postmenopausal women, and the prognostic value of these changes has not been investigated. Accordingly, the aim of the present study was to assess whether antihypertensive treatment is effective in modifying endothelial dysfunction in postmenopausal women and whether an improvement of FMD in response to treatment, as an expression of a reversible endothelial dysfunction, could provide a more favorable prognosis in the studied population.

METHODS

Subjects. Postmenopausal patients with newly diagnosed mild-to-moderate hypertension (stages 1 and 2, according to the Fifth Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure [JNC-V]) (28) and impaired FMD were eligible for the study.

Menopause was defined by the absence of menstruation for at least 12 months or by the dosage of hormone serum level (follicle-stimulating hormone >40 IU/l and 17 beta-estradiol <110 pmol/l).

Hypertension was diagnosed on the basis of two consec-

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Abbreviations and Acronyms

BAD = brachial artery diameter
BP = blood pressure
FMD = flow-mediated dilation, flow-mediated vasodilation
JNC-V = Fifth Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure
NO = nitric oxide
t-PA = tissue-type plasminogen activator
u-PA = urokinase-type plasminogen activator

utive measurements at an interval of one week after an initial high reading. Blood pressure (BP) measurements were performed in accordance with recommendations from the American Society of Hypertension (29).

The patients' history, physical examination, 12-lead electrocardiogram, and echocardiogram were used to exclude past or present heart disease.

From September 1994 to June 1996, 400 consecutive women were enrolled. All gave written, informed consent to participate in this prospective study, which had been approved by the Science and Ethic Committee of our institution.

Study protocol. The study consisted of two parts: 1) high-resolution ultrasonography of the brachial artery at rest and after six months; and 2) follow-up. Enrolled postmenopausal women underwent a basal study of the brachial artery. A second evaluation was scheduled for six months later. In the interim, patients were to receive optimal antihypertensive treatment. All women were advised to modify their life-style habits according to the recommendations of the JNC-V (28). If these measures were unable to reduce BP, pharmacologic therapy was initiated. Therapies were assigned to maintain systolic BP <140 mm Hg and diastolic BP <90 mm Hg. Patients were visited every four weeks. The choice of the antihypertensive drug used was at the discretion of the study investigators, and was made on the basis of a step-by-step approach when the BP values were unsatisfactory (>140/>90 mm Hg). Women were excluded from the study for the following reasons: hypertension greater than stage 2 (28) at admission; a failure to achieve a BP <140/<90 mm Hg after six months of treatment; an improbability of remaining geographically accessible for study visits for at least four years; or a current participation in other ongoing clinical trials.

Ultrasound studies of the brachial artery. An ultrasound study of the brachial artery was performed in all patients at the entry evaluation and after six months by means of a Acuson 128 XP/10 mainframe (Acuson, Mountain View, California) with a 7.0-MHz linear-array transducer. Images were stored on a super VHS videotape recorder for further analysis.

The technique for assessing brachial artery FMD has been described in detail elsewhere (30–33). Briefly, FMD was assessed in a subject's right arm in the recumbent position after a 15-min equilibration period in a temperature-controlled room (22 to 25°C). Brachial artery diameter (BAD) was measured by B-mode ultrasound images at end diastole. The artery was longitudinally imaged ~5 cm proximal to the antecubital crease, where the clearest image was obtained and BAD was measured. After the baseline resting scan, a pneumatic tourniquet placed at the level of the mid forearm (proximal to the target artery) was inflated until no blood flow was detected through the brachial artery with the Doppler probe, and this pressure was held for 5 min. Increased flow was then induced with sudden cuff deflation, and a continuous scan was performed for 1 min. For the reactive hyperemia scan, BAD measurements were taken 45 to 60 s after cuff deflation. Flow-mediated dilation was calculated from the diameters as: (reactive hyperemia − baseline)/baseline × 100% . According to Vogel (22), we considered FMD to be "normal" when the response of the brachial artery was vasodilation >10% relative to baseline and FMD to be "impaired" when vasodilation was <10% or when there was a vasoconstrictive response.

To evaluate the reproducibility of echographic measurements, 100 echographic studies were re-examined by two different investigators (Drs. Modena and Rossi). The studies were selected at random, without knowledge of the patient's identity, clinical information, or previous evaluation results. The interobserver variability for FMD resulted in 0.10 ± 1.69%, and the interobserver linear correlation coefficient was 0.90.

Follow-up. After the second echographic examination, patients were seen in our outpatient clinic at regular intervals (on average, every 12 months). Telephone contact was made every three months to reduce the dropout rate. All cardiovascular events were recorded. All cases were validated by a review of hospital records. The last data elaboration was performed in March 2000. The mean follow-up period was, therefore, 67 months (range 57 to 78).

Statistical analysis. Continuous variables were expressed as the mean value ± SD, and categorical variables as percentages. In the first phase of the study, baseline values and those recorded after six months were compared by repeated measures analysis of variance. The two groups were compared, at the beginning of the follow-up period, by the Student *t* test for unpaired data and the chi-square test with Yate's correction for continuity, as appropriate.

Because the 400 postmenopausal women had different lengths of follow-up, person-years of exposure were calculated for each participant. For individuals who suffered a cardiovascular event, dropped out, or died, exposure was defined as the time between enrollment and the event, the dropout, or the death. For those who did not suffer a cardiovascular event, drop out, or die, exposure was calculated as the time between enrollment and March 2000. Incidence rates of cardiovascular events among patients with persistent impaired FMD and patients with improved FMD

Table 1. Main Demographic Clinical and Brachial Artery Characteristics of the Participants: A Comparison Between Groups

	Group 1 With Persistent Impaired FMD (n = 150)	Group 2 With Improved FMD (n = 250)
Demographic and clinical variables		
Age (yrs)	57 ± 5	56.5 ± 6
Current smokers	32 (21.3%)	54 (21.6%)
Hypercholesterolemia	55 (36.7%)	94 (37.6%)
Diabetes	10 (6.7%)	16 (6.4%)
Body mass index (kg/m^2)	25.5 ± 4.2	25.6 ± 4.6
Heart rate (beats/min)	76 ± 11	78 ± 10
HRT use	20 (13.3%)	33 (13.2%)
Baseline brachial artery characteristics		
BAD (mm)	3.97 ± 0.58	3.98 ± 0.61
FMD (%)	6.9 ± 2.6	6.8 ± 2.7
Six-month brachial artery characteristics		
BAD (mm)	4.01 ± 0.57	3.99 ± 0.55
FMD (%)	7.1 ± 2.5	13.9 ± 2.6*

*$p < 0.0001$ versus group 1. Data are presented as the mean value ± SD or number (%) of patients.

BAD = brachial artery diameter; FMD = flow-mediated dilation; HRT = hormone replacement therapy.

Table 2. Blood Pressure Values at Baseline and After Six Months of Treatment and the Antihypertensive Regimen: A Comparison Between Groups*

	Group 1 With Persistent Impaired FMD (n = 150)	Group 2 With Improved FMD (n = 250)
Baseline blood pressure (systolic/diastolic)	154 ± 14/94 ± 7	155 ± 13/94 ± 8
Blood pressure after 6 months (systolic/diastolic)	132 ± 13/83 ± 8	133 ± 14/82 ± 8
Regimen		
Single-drug therapy	70 (46.7%)	114 (45.6%)
Two antihypertensive drugs	43 (28.7%)	76 (30.4%)
Three or plus antihypertensive drugs	27 (18.0%)	44 (17.6%)
Nonpharmacologic treatment	10 (6.6%)	16 (6.4%)
Antihypertensive used drugs†		
ACE inhibitors	85 (56.7%)	139 (55.6%)
Calcium channel blockers	47 (31.3%)	79 (31.6%)
Beta-blockers	37 (24.7%)	70 (28%)
Thiazide diuretics	53 (35.3%)	94 (37.6%)
Others	12 (8.0%)	21 (8.4%)

*The results of all comparisons were not significant. †Alone or combined. Data are presented as the mean value ± SD or number (%) of patients.

ACE = angiotensin-converting enzyme; FMD = flow-mediated dilation.

were computed as the number of cardiovascular events divided by the total exposure time for each group. The relative risk of a cardiovascular event was computed as the ratio of the incidence rates among patients with persistent impaired FMD divided by the incidence rate among those with improved FMD. Adjusted estimates of risk were computed by use of the Poisson regression model, which controlled for age, diabetes mellitus, smoking habits, serum cholesterol, body mass index, baseline systolic and diastolic BP, and the BP changes from baseline to six-month visit.

Event-free survival was estimated by the product-limit Kaplan-Meier method. Differences between survival curves were tested with the log-rank chi-square statistic. All probability values are two-tailed. A p value <0.05 was considered statistically significant.

RESULTS

Patients. The study included 400 consecutive postmenopausal women with mild-to-moderate hypertension and impaired FMD (FMD ≤10% relative to baseline). At the second evaluation (six months after antihypertensive treatment), FMD had not significantly changed (FMD ≤10% relative to baseline) in 150 (37.5%) of 400 women (group 1; persistent impaired FMD), whereas it had significantly improved (FMD >10% relative to baseline) in the remaining 250 patients (62.5%) (group 2; improved FMD). Baseline demographics, clinical characteristics, and brachial artery characteristics of the two study groups are shown in Table 1. The antihypertensive regimens and proportion of subjects who received a life-style modification only, angiotensin-converting enzyme, calcium channel blockers, beta-blockers and thiazide diuretics, alone or combined, or other drugs are shown in Table 2.

Outcome. During the study period, there were five deaths, all from noncardiac causes (2 accidental and 3 from a neoplasm). There was no difference in the dropout rate: three patients in group 1 and two patients in group 2 (p = NS). Overall, there were 47 cardiovascular events that required hospitalization. All causes of hospitalization are reported in Table 3. Thirty-two (21.3%) of 150 women experienced a nonfatal cardiovascular event in group 1 (persistent impaired FMD) (3.50 events per 100 patient-years), versus 15 (6.0%) of 250 women in group 2 (improved FMD) (0.51 events per 100 patient-years) ($p < 0.0001$) (Table 4 and Fig. 1).

DISCUSSION

The present study demonstrates that in the majority of hypertensive postmenopausal women, a significant improvement in FMD may be obtained after six months of optimized therapy in the majority, and it also clearly

Table 3. Nonfatal Cardiovascular Events Required Hospitalization Recorded During Follow-Up (Mean 67 Months [Range 57 to 78 Months])

	Group 1 With Persistent Impaired FMD (n = 150)	Group 2 With Improved FMD (n = 250)
Acute pulmonary edema	11 (7.3%)	5 (2.0)*
Transient ischemic attack	16 (10.7%)	7 (2.8)†
Ischemic stroke	5 (3.3%)	3 (1.2%)

*$p < 0.001$ versus group 1. †$p < 0.0001$ versus group 1. Data are presented as the number (%) of patients.

FMD = flow-mediated dilation.

Table 4. Incidence Rates of Cardiovascular Events Among 400 Postmenopausal Women

Group	Events, n/N (%)	Person-Years	Incidence Rates*	Incidence Rates Ratio† (Crude)	p Value	Incidence Rates Ratio† (Adjusted)	p Value
Total cohort	47/400 (11.7%)	3,845.2	1.22	—	—	—	—
Group 1 (persistent impaired FMD)	32/150 (21.3%)	912.7	3.50	6.86‡	<0.0001	7.28§	< 0.0001
Group 2 (improved FMD)	15/250 (6.0%)	2,932.5	0.51	Referent	Referent	Referent	Referent

*Incidence rates calculated per 100 person-years of exposure. †Incidence rate ratios were derived from the comparison of the incidence rates between group 1 (persistent impaired FMD) and group 2 (improved FMD). Group 2 represents the referent group. ‡The 95% confidence interval was from 2.93 to 13.72. §The 95% confidence interval was from 3.79 to 14.21.
FMD = flow-mediated dilation.

identifies patients with a more favorable prognosis. Conversely, a higher risk of nonfatal cardiovascular events can be predicted by a noninvasive variable: a lack of change in FMD.

Comparison with previous studies. During the last 10 years, we have observed an increasing volume of reports on the role of endothelial dysfunction in different clinical settings (7–9,19–21,34–36). In fact, it has been demonstrated that endothelial dysfunction itself represents the primum movens in the process of atherosclerosis and vascular remodeling, which are the causes of subsequent development of clinically relevant cardiovascular diseases (22,23,37–39). Therefore, several studies have been published on the role of different classes of drugs (6–9,20–24) or particular diets (40,41) that could be related to a modified or reversible endothelial dysfunction, with the possibility that such interventions, if instituted preventively, might modify the subsequent risk of cardiovascular disease. A recent Medline research shows that, in the year 2000 only, more than 500 articles were published on the role of different therapeutic interventions aimed at influencing endothelial function. Those data reveal the enormous interest in the endothelium as the "new target for cardiovascular therapeutics" (42).

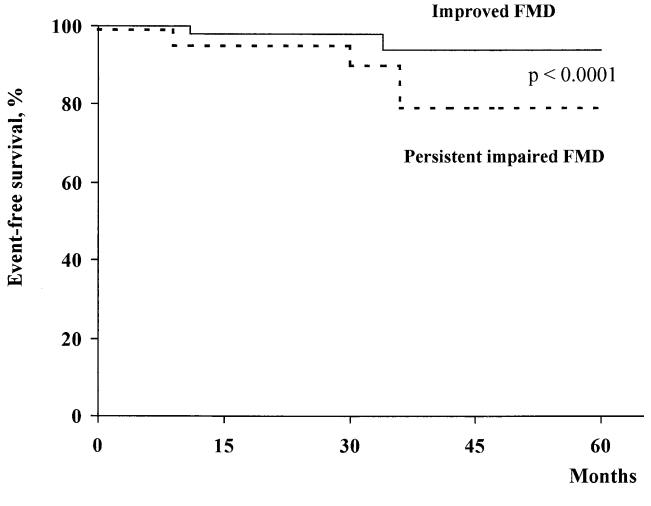

Figure 1. Cumulative survival rates, free of hospital admission, for cardiovascular events in the two study groups, according to persistent impaired (group 1 = **dashed line**) or improved (group 2 = **dotted line**) endothelium-derived FMD.

Nevertheless, the clinician is nowadays principally interested in the prognostic role of reversible endothelial dysfunction and in the clinical relevance of an improvement in endothelial function. In other words, an important question for the clinician is how much modification of FMD is predictive of subsequent risks of cardiovascular events. The present study represents the only one, to the best of our knowledge, focused on the prognostic role of reversible endothelial dysfunction. The results are evident: there is a significantly lower incidence of events in a hypertensive population in which therapy restores normal FMD, in comparison with hypertensive women in which therapy does not influence an abnormal FMD (i.e., persistence of endothelial dysfunction is predictive of future cardiovascular events).

Mechanisms. It is really difficult, in our opinion, to explain the relationship between persistent endothelial dysfunction and the increased risk of clinical events. Our study does not claim to satisfy the question clearly, because, for instance, it is possible that several (still unknown) molecular processes could be implied. We may only hypothesize about some mechanisms. First of all, we noticed a higher rate of transient ischemic attacks in the group with persistent endothelial dysfunction. It is reasonable that there is a close relationship between the two phenomena. The endothelium has, in fact, an important antithrombotic role. Nitric oxide and prostacyclin act synergistically to prevent platelet adhesion and aggregation (43–45). The regulation of fibrinolysis is another important function of the normal endothelium. The two principal determinants of fibrinolysis are: 1) tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA), which promote fibrinolysis; and 2) plasminogen activator inhibitor type 1, which inhibits t-PA and u-PA and enhances formation of thrombi. In normal blood vessels, a basal level of t-PA is secreted, which prevents thrombus formation in the absence of vascular injury (46). In the presence of endothelial dysfunction, enhanced platelet deposition, thrombus formation, and inhibition of vasodilation may explain the high incidence of ischemic episodes in the cerebral vascular territory (47).

A possible interesting mechanism can be hypothesized, taking into account the difference between groups regarding the incidence of acute pulmonary edema, which has a

heightened relevance in the group with permanently impaired endothelial dysfunction. It is well known that pulmonary edema in hypertensive patients is characterized by normal systolic function and is accompanied by a sudden increase in peripheral arterial resistance and, therefore, BP values (48,49). Flow-mediated dilation is altered in patients with essential hypertension, and a reduced response to the muscarinic agonist has been demonstrated in humans (13,50). The generalized decrease in this response suggests a dysfunction mediated by a reduced production or effect of NO (51,52). Given the large increase in arterial resistance observed in animals and humans after inhibition of NO synthesis (51,53–55), it is possible that endothelial dysfunction contributes to the increase in BP values observed during acute pulmonary edema.

Study limitations. A limitation of this study consists in the lack of fatal cardiovascular events, which might be explained by the relatively young age of the women (mean age 56.7 years), the short interval between menopause and the time of enrollment into the study (mean 5.2 years), the low prevalence of risk factors other than hypertension, and the absence of previous cardiovascular events. Consequently, our results need further confirmation in large observational studies that should also include male patients.

Clinical implications. An improvement of initially impaired FMD, obtained with optimized antihypertensive therapy, provides a more favorable prognosis in hypertensive postmenopausal women. The present study indicates that no improvement in endothelial function in hypertensive postmenopausal women relates to an increased risk of cardiovascular events when compared with improved endothelial function and, therefore, suggests a clinical usefulness for periodic measurements of FMD. Patients who cannot achieve normalization of FMD should be considered at high risk of subsequent cardiovascular events, and a more aggressive therapeutic approach is justified in these patients.

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