

Flow-Mediated Vasodilation and the Risk of Developing Hypertension in Healthy Postmenopausal Women

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OBJECTIVES	This study provided the opportunity to assess the relationship between endothelial vasomotor function and incidence of hypertension in a cohort of postmenopausal women.
BACKGROUND	Both menopause and hypertension are associated with endothelial dysfunction and are well-known risk factors for atherosclerotic-related disease.
METHODS	We conducted a prospective cohort study that began in 1996 on 952 apparently healthy postmenopausal women, age 53 ± 5 years (range 44 to 60 years), with initially normal levels of blood pressure and no history of hypertension. All participants were followed up for a mean period of 3.6 ± 0.7 years (range 0.5 to 6.9 years). Endothelial function was measured as flow-mediated dilation of the brachial artery using high-resolution ultrasound.
RESULTS	During follow-up 112 women developed hypertension. The adjusted relative risk for women with flow-mediated dilation of 3.5 or less (lowest quartile) was 5.77 (95% confidence interval 4.34 to 8.10) versus women with flow-mediated dilation of 5.5 or greater (highest quartile, referent). Each one-unit decrease of flow-mediated dilation was associated with a significant 16% (95% confidence interval 12% to 33%) increase in the multiple-adjusted relative risk of incident hypertension.
CONCLUSIONS	These prospective data indicate a significant increase in the relative risk of hypertension with each unit decrease of flow-mediated dilation that is independent of age and baseline systolic and diastolic pressure values. This could suggest that an impaired endothelial vasomotor function precedes and predicts the future development of hypertension in postmenopausal women. (J Am Coll Cardiol 2004;44:1636–40) © 2004 by the American College of Cardiology Foundation

Cardiovascular disease is the leading cause of death in women. Premenopausal women have significant reduced risk of cardiovascular events compared with men of similar age, although the incidence of events increases greatly after menopause (1). The postmenopause is a physiological condition that is known to be associated with endothelial dysfunction, due to a lack of estrogen, that is typical in this phase of a woman's life (2,3). There is considerable evidence that the impairment of endothelial function predicts the development of atherosclerosis (4).

In industrialized countries blood pressure (BP) increases after menopause in women such that the prevalence of hypertension becomes higher in women than in men (5). Endothelial dysfunction is common in hypertensive postmenopausal women (6–10), and hypertension also increases the incidence of atherosclerotic-related diseases (11,12).

Because endothelial dysfunction is also present in non-hypertensive postmenopausal women (2,13), it is not clear

whether endothelial dysfunction is actually a consequence or, rather, the cause of hypertension. This study provided the opportunity to assess prospectively the association between endothelial function, evaluated by ultrasound, and the incidence of hypertension among apparently healthy, normotensive postmenopausal women.

METHODS

Study overview and protocol. The patients enlisted in our study were selected from women who had been referred to the "Bene Essere Donna" center, an institution dedicated to the study, prevention, and treatment of menopause-related disorders. This service, located in a third-level university hospital, is open to all women providing they are in their postmenopausal period (postmenopausal status was defined as the absence of menstruation for at least 6 months and/or by a follicle-stimulating hormone blood level >40 IU/l and 17 beta-estradiol levels <120 pmol/l) and are ≤ 60 years of age. These women, who are initially drawn to the center through local media advertising, have free access and can make queries or obtain advice about particular symptoms they are having by fixing an appointment beforehand. Between March 1, 1996, and July 31, 2003, we assessed 1,682 women.

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Abbreviations and Acronyms

Angiotensin II	Angiotensin II
Angiotensin type 2 receptors	Angiotensin type 2 receptors
Brachial artery diameter	Brachial artery diameter
Blood pressure	Blood pressure
Flow-mediated dilation	Flow-mediated dilation
Nitric oxide	Nitric oxide
Plasminogen activator inhibitor 1	Plasminogen activator inhibitor 1

Physical examination variables measured at baseline included body weight, height, waist circumference, and systolic and diastolic BP. Blood pressure was measured by a physician with a mercury sphygmomanometer and cuff adapted to arm circumference, with the subject sitting for at least 10 min. The appearance of Korotkoff sounds was taken to be the systolic, and their point of disappearance (phase V) the diastolic BP. The average of three measurements was considered for the analysis. Patient history, 12-lead electrocardiogram, and echocardiogram were used to exclude past or present heart disease. At baseline each participant had fasting blood tests for levels of glucose, total cholesterol, and triglycerides. Participants provided questionnaire data concerning lifestyle practices and potential risk factors for cardiovascular disease. The exclusion criteria of the study were hypertension (BP $\geq 140/90$ mm Hg or use of antihypertensive medications), hyperlipidemia (total cholesterol plasma levels ≥ 200 mg/dl and/or triglyceride levels ≥ 170 mg/dl or use of hypolipemic drugs), smoking habits, history of diabetes or use of hypoglycemic drugs or a fasting blood glucose ≥ 126 mg/dl, obesity (body mass index ≥ 30 kg/m²), history of angina, previous myocardial infarction, previous stroke or transient ischemic attack, and previous or current use of hormone replacement therapy.

A total of 952 healthy, normotensive postmenopausal women satisfied the criteria, with a mean age of 53 ± 5 years (range 44 to 60 years). The results were finally elaborated at the end of December 2003, when the last women to enroll had been followed for at least 0.5 years; the mean follow-up was 3.6 ± 0.7 years (range 0.5 to 6.9 years). All participants gave their written informed consent to participate in this study, which had been approved by the science and ethics committee of our institution.

Ultrasound studies of the brachial artery. An ultrasound study of the brachial artery was performed in all participants at baseline by means of an 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 flow-mediated dilation (FMD) has been described in detail elsewhere (14,15). Briefly, FMD was assessed in the subject's right arm in the recumbent position after a 15-min equilibration period in a temperature-controlled room (22°C to 25°C). Each subject had fasted the previous night for at least 12 h. The artery was longitudinally imaged approximately 5 cm

proximal to the antecubital crease, and brachial artery diameter (BAD) was measured at end-diastole. After the baseline resting scan, a pneumatic cuff 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 percent.

To evaluate the reproducibility of echographic measurements, 100 studies were reexamined by two different investigators (M.G.M. and R.R.). These exams were selected at random, without knowledge of the patient's identity, clinical information, or previous evaluation results. The interobserver variability for percent of FMD resulted in $0.10 \pm 1.69\%$.

Definition and ascertainment of cases. All women were seen in our outpatient clinic at regular interval (every six months). Telephone contact was used every three months to reduce the dropout rate. At baseline and at every six-month follow-up visit women underwent an interview, examination, and BP measurements. New cases of hypertension were identified in the following ways: 1) self-reported use of antihypertensive medications; 2) self-report of physician diagnosis; 3) comparison of systolic BP values ≥ 140 mm Hg and/or diastolic BP values ≥ 90 mm Hg during the visit. If this latter condition was encountered, we took steps to check the BP, before diagnosing hypertension, on two further occasions: the first after one week and the second after one month from the initial anomalous measurement. Only in cases where these values remained persistently high (≥ 140 mm Hg for systolic BP and/or ≥ 90 mm Hg for diastolic BP) was hypertension diagnosed. For individuals classified by physician diagnosis or medication use, date of hypertension onset was considered to be the midpoint between the last visit when a woman was not hypertensive and the first visit when a woman was hypertensive. For those diagnosed through the serial measurement of BP values, the onset date of hypertension was considered to be that when hypertension was first encountered. All hypertensive patients were then contacted by telephone to avoid the possibility that a false diagnosis of hypertension could invalidate the results of the study.

Statistical analyses. Continuous variables are presented as mean values ± 1 SD, and categorical variables as percentages. Differences in baseline characteristics between groups were examined by analysis of variance and the chi-square test when appropriate. We used a Cox proportional hazards regression model to analyze the association between endothelial function and incident hypertension. Person-time was calculated from enrollment until the date of hypertension onset, death, dropout, or the study's end, whichever occurred first. The percent of FMD was evaluated in two

Table 1. Baseline Characteristics of the Study Participants, According to the Quartiles of Flow-Mediated Dilatation

	4 ^o Quartile	3 ^o Quartile	2 ^o Quartile	1 ^o Quartile
Levels of FMD, %	≥5.5	4.3-5.4	3.6-4.2	≤3.5
FMD (median), %	6.2	4.9	3.8	3.1
BAD, mm	4.01 ± 0.56	3.99 ± 0.57	3.97 ± 0.58	3.98 ± 0.60
Demographic, anthropometric, and clinical parameters				
Age, yrs	54 ± 5	52 ± 6	52 ± 4	54 ± 6
BMI, kg/m ²	25.9 ± 3.1	26.1 ± 3.0	26.2 ± 2.8	26.0 ± 2.9
Waist circumference, %	84 ± 13	82 ± 13	80 ± 16	81 ± 14
Caucasian, %	100 (n = 238)	100 (n = 238)	100 (n = 238)	100 (n = 238)
Education, yrs	14 ± 3	13 ± 4	13 ± 5	13 ± 5
Living in urban areas, %	79.8 (n = 190)	78.9 (n = 188)	80.6 (n = 192)	76.9 (n = 183)
History of hypertension, %	25.2 (n = 60)	27.3 (n = 65)	24.3 (n = 58)	23.1 (n = 55)
Systolic BP, mm Hg	124 ± 14	128 ± 112	125 ± 13	126 ± 13
Diastolic BP, mm Hg	82 ± 8	83 ± 7	83 ± 8	82 ± 7
Heart rate, beats/min	72 ± 10	73 ± 9	75 ± 7	70 ± 9
Time from menopause, months	45 ± 10	32 ± 11	33 ± 10	44 ± 12
Exercise, times/week				
Never, %	46.2 (n = 110)	47.0 (n = 112)	48.3 (n = 115)	47.8 (n = 114)
1-2, %	39.6 (n = 94)	40.9 (n = 97)	37.5 (n = 89)	43.0 (n = 102)
≥3, %	14.2 (n = 34)	12.1 (n = 29)	14.2 (n = 34)	9.2 (n = 22)
Alcohol consumption				
Never, %	11.7 (n = 28)	10.1 (n = 24)	13.8 (n = 33)	14.7 (n = 35)
≥1 drink per day, %	3.9 (n = 9)	3.4 (n = 8)	5.2 (n = 12)	4.3 (n = 10)
2-6 drinks per week, %	47.0 (n = 112)	49.1 (n = 117)	48.3 (n = 115)	49.5 (n = 118)
1-5 drinks per month, %	37.4 (n = 89)	37.4 (n = 89)	32.7 (n = 78)	31.5 (n = 75)
Biochemical profile				
Total cholesterol, mg/dl	189 ± 17	185 ± 15	190 ± 17	188 ± 19
Triglycerides, mg/dl	146 ± 17	150 ± 15	148 ± 19	154 ± 16
Glucose, mg/dl	86 ± 9	87 ± 8	87 ± 7	85 ± 8

Data are expressed as mean ± 1 SD or as a percentage. Results of all comparisons were non-significant.
BAD = brachial artery diameter; BMI = body mass index; BP = blood pressure; FMD = flow-mediated dilatation.

ways: divided into quartiles and as a continuous term. We tested both the proportional hazard and linearity assumptions of the association between percent of FMD and hypertension and found no violations. We computed crude, age-adjusted, baseline systolic and diastolic BP-adjusted, and multiple-adjusted hazard ratios (and 95% confidence intervals) as a measure of the relative risks of incident hypertension for decreasing percent of FMD, with the highest quartile as the referent. We used an ordinal variable (the median value of percent of FMD in each quartile) to test for trend in risk across FMD quartiles, using the Mantel-Haenzsel extension test (16,17). Adjusted estimates of risk were calculated by the multiple regression model, which controlled for a range of potential confounders selected a priori, including age (continuous), family history of hypertension (yes/no), baseline values of systolic and diastolic BP (continuous), body mass index (continuous), waist circumference (continuous), duration of postmenopausal period (continuous), years of education (continuous), alcohol consumption (never, ≥1 drink per day, 2 to 6 drinks per week, 1 to 5 drinks per month), and physical activity (never, 1 to 2 times per week, ≥3 times per week). Two-tailed p values <0.05 were considered significant.

RESULTS

During 3.6 ± 0.7 years (3,432 person-years) of follow-up, 112 incident cases of hypertension occurred. Of these, 58 of

112 (51.8%) were diagnosed by personal physician, 41 of 112 (36.6%) were diagnosed at our clinic, and 13 patients (11.6%) stated that they were taking antihypertensive drugs. All hypertensive patients were contacted by telephone between March 15 and 31, 2004. All of these patients were taking at least one antihypertensive medication, and 84 of them (75%) were following a therapy involving at least two such drugs.

During follow-up three patients died, all of non-cardiac related causes (one accident and two neoplasm), and we lost contact with 4 patients, making a total of 7 out of 952 patients (0.007%).

Table 1 gives the baseline characteristics of the participants, according to percent of FMD quartiles. There were no significant differences between groups regarding all investigated parameters.

The relative risks of hypertension according to percent of FMD quartiles are summarized in Table 2. The relative risk decreased steadily across percent of FMD categories compared with the referent quartile (FMD ≥ 5.5%) (Fig. 1). Adjustment for age, baseline BP values, and various confounders attenuated the relative risk only slightly.

When percent of FMD was examined as a continuous variable, each one-unit decrease of percent of FMD was associated with a significant 16% (95% confidence interval 12% to 33%) increase in the multiple-adjusted relative risk of incident hypertension.

Table 2. Crude, Age-, Baseline Systolic and Diastolic Blood Pressure Values-, and Multiple-Adjusted Relative Risk of Incident Hypertension by Quartiles of Flow-Mediated Dilation

	4 ^o Quartile (Referent)	3 ^o Quartile	2 ^o Quartile	1 ^o Quartile
Levels of FMD, %	≥5.5	4.3-5.4	3.6-4.2	≤3.5
Incident hypertension (no. of cases) person-yrs	9	19	35	49
	862	910	858	802
Incidence rate (no. of cases per 1,000 person-yrs)	10.4	20.9	40.8	61.1
Relative risk, crude (95% CI)	1.00	2.01 (1.77-3.54)	3.92 (2.85-5.21)	5.87* (4.88-9.06)
Relative risk, age-adjusted (95% CI)	1.00	1.97 (1.70-3.48)	3.39 (2.78-4.90)	5.86* (4.31-8.12)
Relative risk, baseline SBP and DBP-adjusted (95% CI)	1.00	1.95 (1.80-3.55)	3.56 (2.90-5.11)	5.80* (4.30-7.88)
Relative risk, multiple†-adjusted (95% CI)	1.00	1.92 (1.62-3.55)	3.00 (2.43-4.29)	5.77* (4.34-8.10)

*p < 0.0001 test for linear trend. †Multiple regression model adjusted for age (continuous), family history of hypertension (yes/no), baseline values of systolic and diastolic BP (continuous), body mass index (continuous), waist circumference (continuous), duration of postmenopausal period (continuous), years of education (continuous), alcohol consumption (never, ≥ 1 drink per day, 2-6 drinks per week, 1-5 drinks per month), and physical activity (never, 1-2 times per week, ≥ 3 times per week).

CI = confidence interval; DBP = diastolic blood pressure; FMD = flow-mediated dilation; SBP = systolic blood pressure.

DISCUSSION

Our study demonstrates that in healthy, normotensive postmenopausal women, endothelial-dependent vasodilation is a parameter able to significantly predict the future development of hypertension independently of age, baseline BP, and numerous other well-known risk factors. In our opinion, this ability is very important because it radically changes the way of considering endothelial dysfunction. We are used to explaining endothelial dysfunction as a consequence of the endothelium's exposure to damaging factors: high BP levels, high lipid concentrations, high blood glucose levels, smoking, and so on (response-to-injury theory). Our data radically overturn the problem because they suggest that endothelial dysfunction may be the cause (or at least a significant cause) of the development of hypertension.

Although our data support etiological associations, at this time explicit mechanisms remain speculative and require further study. Some hypotheses for our results warrant further discussion. First, it is well known that percent of

FMD induced by reactive hyperemia is endothelium-dependent (18,19); in other words, it depends on the ability of endothelium to produce vasodilator-endowed substances, mainly nitric oxide (NO). It is well demonstrated that postmenopausal status is associated with significant reduced arterial NO activity (20). It is reasonable that our patients with a lower level of percent of FMD had a higher degree of endothelial dysfunction, essentially meaning a lower production of NO. There are numerous reports on the fact that a chronic inhibition in the production of NO is able to cause an increase in peripheral resistance, an impaired renal ability to excrete sodium (all mechanisms that contribute in increasing BP), and progressive renal damage in experimental hypertension (21-23). Even in humans, a gradual inhibition in the production of NO is associated with an increase in arterial resistance (24). Furthermore, an important correlation between endothelial dysfunction and the development of hypertension is mediated by angiotensin II (AII). It is well known that in postmenopausal women plasma renin activity and the consequent production of AII is significantly higher with respect to the premenopausal period (25). Angiotensin II, through type 2 angiotensin receptors (AT2), leads to vasodilation. It is also known that AII vasodilatory action is controlled by the NO produced by the endothelium (26). It is therefore reasonable to assume that a normal endothelial function, with a consequent normal production of NO, plays a highly limiting role in the development of hypertension.

Another consequence of endothelial dysfunction is the increased production of prothrombotic activity substances, the most important of which being plasminogen activator inhibitor 1 (PAI-1). Fibrinolytic activity is in fact primarily mediated by the balance between the levels of tissue plasminogen activator and PAI-1, both of which are synthesized by the endothelium (27). Therefore, endothelium injury induces an imbalance in fibrinolysis (28,29). So it is reasonable to assume that in case of endothelial dysfunction the production of PAI-1 increases even in postmenopausal women. It is known that hypertensive patients have PAI-1 values that are notably higher with respect to normotensive

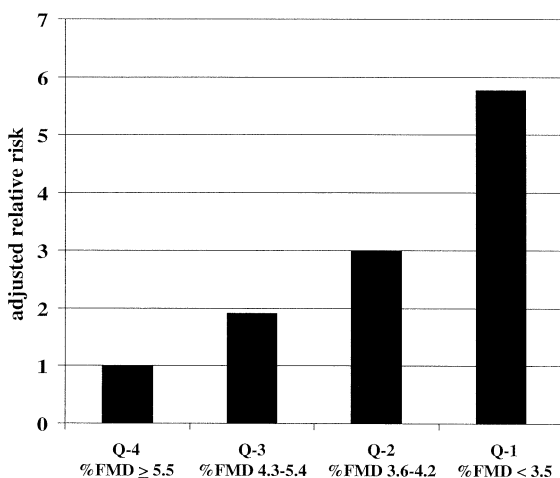


Figure 1. Relative risk of developing hypertension in relation to the percent of flow-mediated dilation (FMD) quartiles. The fourth quartile must be considered as the referent one. p < 0.0001 for trend. **Numbers in the bars** represent the incidence rate (new cases of hypertension per 1,000 person-years). Q = quartile.

patients (30,31); moreover, the relationship between thrombosis and hypertension is so tight that in Framingham's cohort a significant correlation between BP values and PAI-1 levels was clearly demonstrated (32).

Limits of the study. Our study has several strengths, including the prospective design, the large sample of examined women, the relatively homogeneous nature of the cohort, and the ability to exactly determine the time of enrollment and hypertension onset.

The most important limit of the study is that it is a non-randomized, strictly observational study that shows an interesting association between percent of FMD and risk of developing hypertension, which may indicate that some as-yet-undiscovered pathophysiological alteration drives both processes or may be causal. For these reasons our findings should be viewed with caution. Another limiting factor to our study was the fact that all participants were relatively young and free from cardiovascular risk factors and cardiac pathologies. The results we obtained, therefore, are applicable only to a limited number of postmenopausal women, not to all; nor, for that matter, are they applicable to men.

Clinical implications. The perspectives of these data may be of great clinical impact. Locating an impaired percent of FMD permits us to recognize those patients that are at high risk for the development of hypertension. Such patients should therefore be persuaded to alter their lifestyle with the aim of reducing this risk. A close follow-up and aggressive management of other known cardiovascular risk factors would be justified in these subjects.

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