Usefulness of Brachial Artery Flow-Mediated Dilation to Predict Long-Term Cardiovascular Events in Subjects Without Heart Disease

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Endothelial dysfunction is considered an important prognostic factor in atherosclerosis. To determine the long-term association of brachial artery flow-mediated dilation (FMD) and adverse cardiovascular (CV) events in healthy subjects, we prospectively assessed brachial FMD in 618 consecutive healthy subjects with no apparent heart disease, 387 men (63%), and mean age 54 ± 11 years. After overnight fasting and discontinuation of all medications for ≥12 hours, FMD was assessed using high-resolution linear array ultrasound. Subjects were divided into 2 groups: FMD ≤11.3% (n = 309) and >11.3% (n = 309), where 11.3% is the median FMD, and were comparable regarding CV risk factors, lipoproteins, fasting glucose, C-reactive protein, concomitant medications, and Framingham 10-year risk score. In a mean clinical follow-up of 4.6 ± 1.8 years, the composite CV events (all-cause mortality, nonfatal myocardial infarction, hospitalization for heart failure or angina pectoris, stroke, coronary artery bypass grafting, and percutaneous coronary interventions) were significantly more common in subjects with FMD ≤11.3% rather than >11.3% (15.2% vs 1.2%, p = 0.0001, respectively). Univariate analysis demonstrated that the median FMD significantly predicted CV events (odds ratio 2.78, 95% CI 1.35 to 5.71, p <0.001). Multivariate analysis, controlling for traditional CV risk factors, demonstrated that median FMD was the best independent predictor of long-term CV adverse events (odds ratio 2.93, 95% CI 1.28 to 6.68, p <0.001). In conclusion, brachial artery median FMD independently predicts long-term adverse CV events in healthy subjects with no apparent heart disease in addition to those derived from traditional risk factor assessment. © 2014 Elsevier Inc. All rights reserved. (Am J Cardiol 2014;113:162−167)

Endothelial dysfunction is a major factor in the development of atherosclerosis,[1−5] hypertension,[6] and heart failure.[7]

Flow-mediated dilation (FMD) of the brachial artery by ultrasound is widely used as a measure of endothelial function.[2,3,8] Vascular endothelial dysfunction is an independent risk factor for cardiovascular (CV) events and provides important prognostic data in addition to the more traditional CV risk factors.[9,10] However, the effect of several classic and nonclassic CV risk factors on endothelial function in healthy subjects has not been clarified. On the basis of the hypothesis that healthy subjects with reduced endothelial function have worse long-term outcome than those with good endothelial function, we aimed to detect the association of peripheral vascular endothelial function, assessed by brachial artery vasoreactivity, and its long-term outcome in healthy subjects with no apparent heart disease.

Methods

The study population was based on 618 consecutive subjects recruited prospectively from the Endothelial Function Assessment Laboratory of the Leviev Heart Center at the Chaim Sheba Medical Center. Inclusion criteria included healthy subjects (i.e., no history of angina pectoris, myocardial infarction, coronary artery bypass grafting surgery, coronary angiography with angioplasty and/or stenting, any lesion >50% of the coronary artery luminal diameter, cerebrovascular accident, or peripheral vascular disease) with normal electrocardiograms and echocardiograms. Exclusion criteria included atrial fibrillation, sinus bradycardia (heart rate <50 beats/min) without pacemaker, sick sinus syndrome, second- or third-degree atrioventricular block, intolerance to nitrates, renal failure with serum creatinine level >3 mg/dl, history of drug or alcohol abuse, chronic liver disease, or refusal to sign the informed consent form. Cardiac risk factors assessed included age, gender, tobacco smoking, diabetes, hypercholesterolemia, hypertension, and body mass index (BMI). Smokers included subjects with current tobacco use. Diabetes was defined as a fasting blood glucose level of >126 mg/dl or treatment with dietary modification or oral hypoglycemic agents at the time of the study. Hypercholesterolemia was defined as a fasting serum total cholesterol level >200 mg/dl or treatment with lipid-lowering medication or dietary modification. Hypertension was defined as a seated systolic blood pressure measurement of >140 mm Hg, diastolic pressure of >90 mm

Table 1
Characteristics of subjects above and below the median brachial flow-mediated dilation (FMD)

Parameter	FMD		p
	≤11.3% (n = 309)	>11.3% (n = 309)	
Age (yrs)	55 ± 11	54 ± 11	0.092
BMI (kg/m^2)	27 ± 4	27 ± 5	0.627
Men	198 (64)	189 (61)	0.454
Hypertension (>140/90 mm Hg)	107 (35)	84 (27)	0.045
Hypercholesterolemia (>200 mg/dl)	80 (26)	78 (25)	0.854
Current smokers	43 (14)	42 (14)	0.907
Oral-treated type 2 diabetes mellitus	15 (5)	12 (4)	0.690
Family history of premature CAD	78 (25)	80 (26)	0.854
Medications			
Aspirin use	15 (5)	13 (4)	0.124
Statin use	80 (26)	78 (25)	0.854
Long-acting nitrate use	3 (1)	2 (0.6)	0.612
Calcium channel blocker use	37 (12)	22 (7)	0.040
Furosemide (Fusid) use	14 (5)	13 (4)	0.844
Spironolactone use	2 (0.6)	3 (0.9)	0.653
Angiotensin-converting enzyme inhibitors	46 (15)	24 (8)	0.005
β Blockers	51 (17)	44 (14)	0.435
Multivitamins	15 (5)	21 (7)	0.303
Fasting blood glucose (mg/dl)	94 ± 14	98 ± 15	0.436
Total cholesterol (mg/dl)	207 ± 22	210 ± 27	0.552
Low-density lipoprotein cholesterol (mg/dl)	126 ± 22	129 ± 27	0.579
Triglycerides (mg/dl)	145 ± 31	151 ± 35	0.597
High-density lipoprotein cholesterol (mg/dl)	48 ± 12	51 ± 14	0.086
Homocysteine (μmol/L)	13 ± 3	13 ± 2	0.242
Systolic blood pressure (mm Hg)	133 ± 12	133 ± 11	0.799
Diastolic blood pressure (mm Hg)	81 ± 10	80 ± 11	0.419
Heart rate at rest (beats/min)	66 ± 10	68 ± 11	0.137
Mean pulse pressure (mm Hg)	57 ± 17	58 ± 17	0.906
Mean arterial pressure (mm Hg)	100 ± 12	99 ± 13	0.538
High-sensitivity C-reactive protein (mg/L)	2.1 ± 1.1	1.8 ± 1.1	0.558
Framingham risk score (% risk/10 yrs)	7.7	7.2	0.786
Baseline brachial artery diameter (mm)	6.02 ± 0.95	5.27 ± 0.88	<0.001
Nitroglycerin-induced dilation (%)	16.8 ± 4.3	17.2 ± 4.0	0.759
FMD	5.1 ± 4.1	18.9 ± 4.3	<0.001

Values are mean ± SD or n (%).

Hg on at least 3 separate occasions, or the presence of such a diagnosis in the past, with the patient being treated with medications or lifestyle modification. BMI was calculated as weight (kilograms) divided by height (square meter).

After an overnight fast and the discontinuation of all medications (for those who were taking any medication) and cigarette smoking for ≥12 hours, a physical examination, brachial artery reactivity testing, and blood tests for measurements of lipids, blood cell count, electrolytes, fasting glucose, homocysteine, and high-sensitivity C-reactive protein were performed. The blood samples were centrifuged immediately for 15 minutes at 3,000/min. The serums were stored at −20°C. All blood samples were tested at the end of the study in the same laboratory and by the same operator who was blinded to the patients' clinical status and endothelial function results. The hospital review board approved the study, and all participants gave written informed consent.

Endothelial function in the form of endothelium-dependent FMD in the brachial artery was measured as previously described.[2,3,8,11–16] Briefly, FMD was assessed by a single ultrasonographer blinded to the subject's clinical status. The test was performed in the subject's left arm while in a recumbent position in a quiet temperature-controlled room (22°C) after a 10-minute equilibration period. Using a 15- to 6-MHz linear array (15-6L HP) ultrasound (HP SONOS 7500 cv system; Agilent Technologies Inc., Andover, Massachusetts), the brachial artery was longitudinally imaged approximately 5 cm proximal to the antecubital crease. An electrocardiogram was monitored continuously, and blood pressure was taken in the right arm each minute throughout the study.

After a 2-minute baseline period, a longitudinal image of 3 cm of vessel without color flow was obtained and frozen for 5 seconds. The image was then unfrozen and switched to a pulse-wave Doppler for 5 seconds at a sweep speed of 50 mm/s. A pneumatic tourniquet (AG101; Hokanson, Bellevue, Washington), placed around the left upper arm proximal to the target artery (upper arm occlusion), was inflated after the baseline phase to 50 mm Hg above the subject's systolic blood pressure (or until no blood flow was observed in the brachial artery by Doppler probe) and held for 5 minutes. Upper arm occlusion was followed by a hyperemic state, which is mainly dependent on metabolic local changes in favor of vasodilating substances, and only

Table 2
Cardiovascular events during follow-up

Event	FMD		p
	≤11.3%, n = 309 (%)	>11.3%, n = 309 (%)	
Mortality	6 (1.9)	1 (0.3)	0.512
Nonfatal myocardial infarction	5 (1.6)	1 (0.3)	0.196
Cerebrovascular accident	4 (1.2)	1 (0.3)	0.065
Chronic heart failure	1 (0.3)	0	0.323
Angina pectoris	19 (6.1)	0	0.206
Coronary artery bypass grafting	3 (0.9)	1 (0.3)	0.130
Percutaneous coronary intervention	9 (2.9)	0	0.153
All events	47 (15.2)	4 (1.2)	<0.0001

Table 3
Multivariate analysis* demonstrating the association of traditional risk factors for coronary artery disease and long-term composite cardiovascular end points in the study population (n = 618)

Risk Factors for CAD	HR	95% CI		p
		Lower	Upper	
Age	1.01	0.984	1.041	0.405
Men	0.37	0.164	0.854	0.020
Hypertension	1.52	0.805	2.870	0.197
BMI	0.95	0.887	1.021	0.166
Hyperlipidemia	0.74	0.305	1.804	0.509
Smoking	1.83	0.630	5.339	0.266
Diabetes mellitus	1.70	0.491	5.939	0.400
Family history of premature CAD	0.24	0.778	1.783	0.176
Median FMD = 11.3%	2.93	1.285	6.688	<0.001

CI = confidence interval; HR = hazard ratio.

* Cox regression analysis.

partially endothelial-mediated. Increased flow was subsequently induced by sudden cuff deflation, followed by a continuous scan at deflation, at 20, 40, 60, 90, and 120 seconds, with frozen and Doppler measurements recorded at similar intervals from baseline.

A second 2-minute baseline scan at rest was recorded to confirm vessel recovery 13 minutes after cuff deflation. Scanning was performed continuously for 5 minutes after administration of a sublingual nitroglycerin (NTG) tablet (Nitrostat, 0.4 mg; Park-Davis, California).

Ultrasound images were recorded on an S-VHS videotape with an SLV-RS7 videocassette recorder (Sony, California). Brachial artery diameter was measured from the anterior to the posterior interface between the media and adventitia ("m line") at a fixed distance. The mean diameter was calculated from 4 cardiac cycles synchronized with the R-wave peaks on the ECG. All measurements were calculated at end-diastole to avoid possible errors resulting from variable arterial compliance. The internal diameter was calculated with PC Prosound software (USC, Los Angeles, California) using a Horita Data Translation Image Processing board (DT2862-60Hz; Mission Viejo, California).[11–13] Percent

FMD and percent NTG were expressed as the percent change relative to that at the initial scan taken at rest. Percent FMD was computed from the formula [(maximum diameter − baseline diameter)/baseline diameter × 100]. Percent FMD, using the maximal brachial artery diameter achieved after cuff deflation, was used as an index of endothelium-dependent dilation; percent dilation was obtained 5 minutes after the administration of NTG represented percent NTG. The intraobserver correlation coefficients for baseline and deflation diameters were 0.99. The absolute error between measurements ranged from 0 to 0.12 mm (for brachial artery diameter) and 0.02% to 2.98% (for FMD). The determination of endothelial function was performed in accordance with published guidelines.[2,3]

All patients were followed by telephonic contact after a mean of 4.6 ± 1.8 years for combined adverse CV end points, which included all-cause mortality, nonfatal myocardial infarction, hospitalization for heart failure or angina pectoris, stroke, coronary artery bypass grafting, and percutaneous coronary interventions, by physicians who were blinded to the patients' baseline clinical status and endothelial function results. All CV events were validated by the review of medical records by senior cardiologists who were blinded to the FMD results. All patients were followed up every 6 months by a telephone call and also by an MD clinic visit. All subjects were registered in the Ministry of Health computerized central registry, in which all hospitalizations, diagnoses, and procedures (including operations, catheterizations, MD/clinic consultations, noninvasive tests, laboratory examinations, pathologic tests, computerized tomography, x-ray, electrocardiography, and so on) were listed, which enabled the use of this register for follow-up, in addition to the telephone calls and clinic visits. In addition, online access to the aforementioned data facilitated verification that all events were well documented. In addition, written medical records were reviewed by cardiologists in the event of any death, hospitalization, and/or angina pectoris. No patient was lost from follow-up.

All the clinical and laboratory data were analyzed using SPSS software (version 17.0, IBM, USA). Chi-square tests were used for the relation between categorical variables, and comparison between the 2 groups (below and above the median FMD) was conducted using an independent *t* test. Multivariate analysis was done by Cox regression for estimating coronary artery disease (CAD) risk factors. The end point was defined as the first occurrence of any CV event, thereby ensuring that no patient was counted twice. A Kaplan-Meier survival curve analysis showing survival until first composite CV end point (all-cause mortality, nonfatal myocardial infarction, hospitalization for heart failure or angina pectoris, stroke, coronary artery bypass grafting, and percutaneous coronary intervention) was performed for subjects with FMD below and above the median, controlling for traditional CAD risk factors (age, gender, lipoproteins, diabetes, smoking, family history, hypertension, and BMI). A logistic regression model, adjusted for age, male gender, and traditional CAD risk factors, was used to demonstrate the effect of time on endothelial function. A cross-validation analysis was achieved by excluding subjects with CV end points. Statistical significance was 2-tailed and determined as p <0.05.
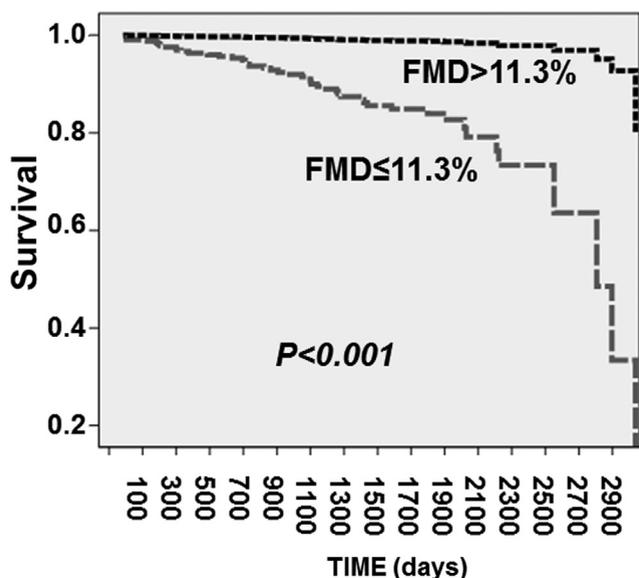
Figure 1. A Kaplan-Meier survival curve showing survival until the first composite adverse CV end point (cardiac mortality, nonfatal myocardial infarction, hospitalization for heart failure or angina pectoris, stroke, coronary artery bypass grafting, and percutaneous coronary interventions) in subjects with FMD above *(dashed line)* and below *(solid line)* the median value of 11.3%, after controlling for traditional risk factors (age, gender, lipoproteins, diabetes, hypertension, and BMI). Patients with FMD below the median had lower survival rate compared with those with FMD above the median (p <0.001).

Results

The study population comprised 618 non-CAD volunteers, 387 men (63%), 231 women (37%), mean age of 54 ± 11 years (range 17 to 81), and BMI of 27 ± 4 kg/m^2. The study population included 191 subjects (31%) with hypertension, 158 (26%) with hypercholesterolemia, 27 (4%) with type 2 diabetes, 85 (14%) current cigarette smokers, and 158 (26%) with a family history of premature CAD. Mean left ventricular ejection fraction was 60 ± 3%, and the study cohort 10-year Framingham risk score was 7.5%.

The mean baseline brachial artery diameter of the study population was 5.65 ± 0.99 mm, mean FMD was 12.0 ± 3.2% (median 11.3%), and mean NTG was 15.1 ± 3.5% (median 15.2%). Subjects were divided into 2 groups: FMD ≤11.3% (n = 309) and >11.3% (n = 309), where 11.3% is the median FMD. The 2 groups were comparable regarding CAD risk factors, lipid levels, fasting glucose levels, homocysteine, high-sensitivity C-reactive protein, blood pressure at rest, and concomitant medications (Table 1). However, hypertension and the use of angiotensin-converting enzyme inhibitors and calcium channel blockers were significantly more common in subjects with FMD below compared with above the median FMD.

In a mean follow-up of 4.6 ± 1.8 years, 48 of 618 patients (7.7%) had 51 composite adverse CV end points (Table 2). Stroke was more common (although not statistically significant) in those with FMD below compared with those with FMD above the median FMD. All composite adverse CV end points were significantly more common in subjects with FMD below than above the median FMD (p <0.001).

Univariate analysis demonstrated that the median FMD significantly predicted CV events (odds ratio 2.78, 95% confidence interval 1.35 to 5.71, p <0.001). In a multivariate analysis using a logistic regression model, we demonstrated that FMD on a first CV adverse event, controlling conventional risk factors for atherosclerosis (odds ratio 2.93, 95% confidence interval 1.285 to 6.688, p <0.001; for FMD below the median) was a significant risk factor beyond all other traditional risk factors for CAD (Table 3). A Kaplan-Meier survival curve demonstrated, after controlling for traditional CAD risk factors, that subjects with FMD below the median value of 11.3% had significantly higher composite CV adverse end points compared with those with FMD above the median (p <0.001; Figure 1).

The baseline brachial artery diameter was larger in the lower FMD group, indicative of preclinical atherosclerosis and early remodeling. However, we did not find any association between baseline brachial artery diameter and prediction of worse outcome. Although FMD was significantly related to the baseline brachial artery diameter, it was not a prognostic predictor (5.6 ± 1% vs 5.3 ± 1%, p = 0.102, respectively).

Discussion

A limited number of studies with relatively small sample size have examined the relation between endothelial dysfunction and prognosis in patients without CV disease (i.e., primary prevention setting). Halcox et al[17] showed that invasively measured coronary endothelial dysfunction is an independent predictor of CV events in patients with and without CAD. Bugiardini et al[18] showed that coronary endothelial dysfunction in women with chest pain, but angiographically normal coronary arteries, is a sign for future development of CAD. Perticone et al[19] showed that endothelial dysfunction, by invasively measuring forearm blood flow, is a marker for future CV events in patients with essential hypertension. Neunteufl et al[20] showed that endothelial dysfunction, as demonstrated by brachial artery FMD, correlates with cardiac events in patients with chest pain but without previous CAD who had undergone coronary angiography. In this study,[20] however, most events were revascularization procedures, so that the predictive value of endothelial dysfunction for these outcomes was lost after controlling for extent of CAD. Furthermore, Yeboah et al[21] demonstrated in a large prospective study comprising 2,792 subject aged 72 to 98 years from the CV Health Study that FMD is a predictor of CV events in a 5-year follow-up period but added very little to the prognostic accuracy of traditional CV risk factors in older adults. Similarly, in the Multi-Ethnic Study of Atherosclerosis, with >3,000 subjects, FMD again predicted future CV events, even after adjustment for the Framingham risk score.[22] In addition, FMD in combination with the Framingham risk score helped to classify CV risk better than FMD or the Framingham risk score alone.[22]

Our present work is in concordance with our previous report[23] on 435 healthy subjects in which FMD was found to be the best independent predictor of long-term (32 ± 2 months) CV adverse event in addition to the traditional CAD risk factor assessment. Furthermore, FMD independently

predicted long-term adverse CV events also in 268 consecutive healthy subjects, without any concomitant medications who were followed for 45 ± 21 months.[24]

Therefore, our present prospective study is in accordance with the previous clinical trials demonstrating that FMD in combination with the Framingham score helped classify CV risk better than FMD or the Framingham score alone.[22] This is not surprising because endothelial dysfunction is a key biologic mechanism by which CV risk factors exert their propensity for atherosclerotic and adverse events.[8] Thus, clinical assessment of brachial artery endothelial function provides valuable independent prognostic information that may play a potential role in the long-term management of subjects with risk factors for atherosclerosis. This body of clinical evidence fits well with current paradigm depicting the endothelium as an organ that integrates signals between the milieu of the vascular wall and vessel lumen. In this context, endothelial function should represent an excellent "barometer" of underlying vascular health as it represents an orchestrated response to the many known and unknown processes that contribute to the development, progression, and clinical expression of atherosclerosis. Indeed, it is plausible that endothelial resistance to the adverse effects of risk factors might be determined by genetic or environmental factors that are currently difficult to quantify using traditional risk assessment models.[25]

The present study has some limitations. (1) Because shear stress is the stimulus for FMD, it seems reasonable to correct for the impact of shear stress on FMD[8,26]; however, in the present study, we did not assess normalized FMD (FMD/shear rate) on cardiovascular prognosis. (2) In the present study, we used the upper arm cuff occlusion for evaluating FMD. When the cuff is placed on the upper part of the arm, reactive hyperemia typically elicits a greater percent change in diameter compared with that produced by the placement of the cuff on the forearm.[2,3,27] This may be due to a greater flow stimulus resulting from recruitment of more resistant vessels or possibly to direct effects of ischemia on the brachial artery. However, upper arm occlusion as used in the present study is technically more challenging for accurate data acquisition as the image is distorted by collapse of the brachial artery and shift in soft tissue.[2,3] Subsequent studies have shown that upper arm occlusion produces a greater degree of brachial vasodilation than lower arm occlusion and has thus been proposed as a more efficient means of evaluating endothelial function.[28,29] Finally, it should be noted that the International Brachial Artery Reactivity Task Force[2] has been unable to reach a consensus as to which technique provides the most accurate or precise data. (3) This study cohort is biased because patients were usually from a better socioeconomic background (those without private insurance are required to pay for the test) and were more educated (awareness of primary care facilities). (4) Stress tests were not performed on all patients; however, the exclusion of CAD was based on clinical examination, electrocardiography, and echocardiography, which were performed on all participants. (5) It should be noted that our study population comprised patients at low CAD risk with an estimated 10-year risk of <10%, and therefore, our data cannot be extrapolated to patients at moderate or high risk for CAD.

Disclosures

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