Clinical Correlates and Heritability of Flow-Mediated Dilation in the Community

The Framingham Heart Study

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Background—Studies in selected samples have linked impaired endothelial function with cardiovascular disease and its risk factors. The clinical correlates and heritability of endothelial function in the community have not been described.

Methods and Results—We examined a measure of endothelial function, brachial artery flow-mediated dilation (FMD), expressed as both percent (FMD%) and actual dilation by ultrasound with the occlusion cuff below the elbow in 2883 Framingham Study participants (52.9% women; mean age, 61 years). A subset of 1096 participants performed a 6-minute walk test before FMD determination. Mean FMD% was 3.3±3.0% in women and 2.4±2.4% in men. In stepwise multivariable linear regression models, FMD% was inversely related to age, systolic blood pressure, body mass index (BMI), lipid-lowering medication, and smoking, whereas it was positively related to female gender, heart rate, and prior walk test. The estimated heritability of FMD% was 0.14. FMD actual dilation findings were similar, except that female sex and BMI were not significantly associated.

Conclusions—Increasing age, systolic blood pressure, BMI, and smoking were associated with lower FMD% in our community-based sample, whereas prior exercise and increasing heart rate were associated with higher FMD%. The estimated heritability of FMD was modest. Future research will permit more complete characterization of the genetic and environmental determinants of endothelial function and its prognostic value in the community. **(*Circulation*. 2004; 109:613-619.)**

Key Words: endothelium ■ epidemiology ■ risk factors ■ genetics

Brachial artery flow-mediated dilation (FMD) serves as a measure of endothelial vasodilator function in humans.[1] Experimental and clinical studies suggest that development of endothelial dysfunction, including reduced NO bioavailability, contributes to the atherosclerosis and pathogenesis of cardiovascular disease (CVD) events.[2] Human studies demonstrate that endothelial dysfunction precedes the development of clinically apparent atherosclerosis in individuals with CVD risk factors such as smoking,[3] hypertension,[4] hyperlipidemia,[5] diabetes mellitus,[5] and obesity.[6] Furthermore, effective treatment of risk factors may reverse endothelial dysfunction.[7] Finally, studies in individuals with risk factors or prevalent CVD have demonstrated that endothelial dysfunction identifies patients at risk for future CVD events.[2,8]

Previous investigations relating risk factors to endothelial dysfunction largely were limited to small, highly selected samples. Our objective was to assess the independent correlates of endothelial function in a large community-based sample.

Methods

The Framingham Offspring Study design has been described elsewhere.[9] Participants in the seventh examination (1998 to 2001) were eligible for the present investigation (n=3539). Exclusion criteria were residence in a nursing home (n=205), mastectomy (n=34), Raynaud disease (n=9), subject refusal (n=83), equipment malfunction/miscellaneous (n=15), predigital capture (n=177), or technically inadequate study (n=133). The Boston University Medical Center Institutional Review Board approved the protocol; participants gave informed consent.

All participants underwent routine medical history, physical examination, and laboratory assessment. Participants were instructed not to eat or drink after 8 PM the previous evening, with the exception of water or decaffeinated black coffee or tea. Cigarette smoking within the prior year and past 6 hours was self-reported. Heart rate and blood pressure (BP) were measured by automatic device

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TABLE 1. Sample Characteristics

	Women (n=1526)	Men (n=1357)
Age, y	61±9	61±10
SBP, mm Hg	122±18	128±17
DBP, mm Hg	67±11	75±10
Heart rate, bpm	65±11	62±11
BMI, kg/m^2	27.6±5.8	28.9±4.6
Total/HDL cholesterol, ratio	3.6±1.2	4.5±1.4
Triglycerides, mg/dL	132±77	144±102
Fasting glucose, mg/dL	101±25	109±29
Diabetes, %	10.4	16.5
Smoking, %	13.8	13.2
Smoking within past 6 hours, %	9.0	8.9
Prevalent CVD, %	8.3	18.3
Hypertension, %	41.3	49.1
Hormone replacement therapy, %	35.8	0
Hypertension medication, %	29.7	37.1
Lipid-lowering medication, %	17.7	24.5
Walk test		
Before brachial testing, %	39.7	36.4
After brachial testing, %	37.8	34.9

Continuous measures, mean±SD; categorical variables, percentages. DBP indicates diastolic blood pressure.

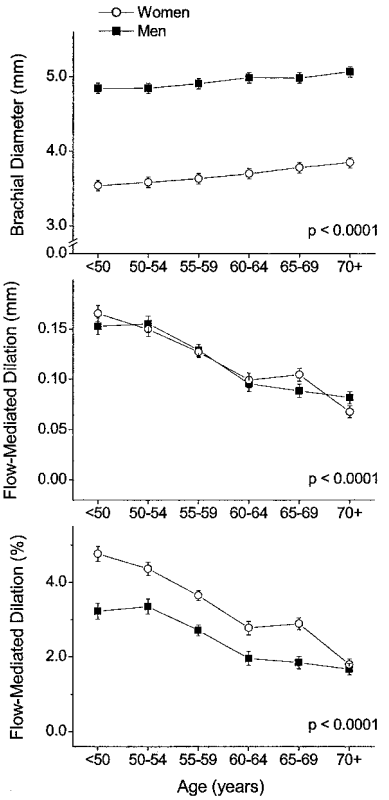

Figure 1. Mean and standard error of brachial measures by sex and 5-year age group; *P* value is for sex-adjusted trend test.

(Dinamap, Critikon, Inc). Diabetes was defined as a fasting glucose ≥126 mg/dL or use of medication. A panel of 3 blinded investigators determined CVD from medical records.[10] The examination included a 6-minute walk test (Bruce protocol stages I and II) in participants without contraindications (known coronary disease, chest pain on test day, or inability to perform test).

Determination of FMD

The brachial artery was imaged with a Toshiba SSH-140A ultrasound system and 7.5-mHz linear-array transducer. Arterial flow was interrupted for 5 minutes by cuff placed on the proximal forearm (Hokanson AG101) at whichever occlusion pressure would be higher: 200 mm Hg or 50 mm Hg+systolic BP (SBP). Using electrocardiographic triggering, end-diastolic images were digitally captured at baseline and for 2 minutes after cuff deflation (Cardiovascular Engineering).

Brachial artery diameter (6- to 10-mm segment) was measured offline using commercially available software (Brachial Analyzer, Medical Imaging Applications) by blinded sonographers. Baseline diameter was calculated as the average diameter from all baseline images measured. The 60-second diameter was calculated as the average of all images measured between 55 and 65 seconds after cuff deflation. FMD induced by reactive hyperemia was expressed as actual FMD ([60-second]−[baseline diameter]=FMDmm) and as relative change from baseline (FMDmm/baseline diameter=FMD%).

Quality-assurance methods included previously described written protocols and measurement variability assessments.[1] Reproducibility results compared the 3 sonographers' measurements of 20 studies separated by 1 year. The intraobserver and interobserver correlation coefficients for baseline and deflation diameters were 0.99. The absolute error between measurements ranged from 0 to 0.12 mm (FMDmm) and 0.02% to 2.99% (FMD%). For both FMDmm and FMD%, the correlation coefficients ranged between 0.78 and 0.92, comparable to reports from other laboratories.[11–13]

Statistical Analysis

Stepwise multivariable regression (SAS REG procedure[14]), with age and gender forced in, was used to select correlates of baseline

TABLE 2. Brachial Artery Measures

		Flow-Mediated Dilation	
	Baseline Diameter, mm	Millimeters	Percent
Mean±SD (range)			
Women	3.69±0.57 (2.19 to 6.10)	0.12±0.10 (−0.11 to 0.53)	3.3±3.0 (−2.8 to 18.0)
Men	4.95±0.64 (2.63 to 7.96)	0.11±0.11 (−0.15 to 0.69)	2.4±2.4 (−3.3 to 14.0)
Correlation			
Baseline diameter	...	−0.12*	−0.31*
FMDmm	...	...	0.96*
Framingham risk score	0.37*	−0.19*	−0.27*

*$P<0.0001$.

TABLE 3. Age- and Sex-Adjusted Models

	Baseline Diameter		Flow-Mediated Dilation			
			Millimeters		Percent	
	β	P	β	P	β	P
Sex, female vs male	−1.26	0.0001	0.002	0.57	0.89	0.0001
Age, 10 y	0.11	0.0001	−0.031	0.0001	−0.84	0.0001
SBP, 20 mm Hg	0.06	0.0001	−0.025	0.0001	−0.66	0.0001
DBP, 10 mm Hg	0.05	0.0001	−0.015	0.0001	−0.39	0.0001
Heart rate, 10 bpm	0.02	0.11	0.002	0.28	0.05	0.29
BMI, 5 kg/m^2	0.16	0.0001	−0.006	0.001	−0.26	0.0001
Total/HDL cholesterol, 1 unit	0.04	0.0001	−0.003	0.07	−0.12	0.002
Triglycerides, 90 mg/dL	0.03	0.003	−0.004	0.02	−0.15	0.002
Fasting glucose, 25 mg/dL	0.05	0.0001	−0.005	0.004	−0.14	0.001
Diabetes	0.14	0.0001	−0.017	0.003	−0.46	0.002
Smoking	−0.08	0.02	−0.008	0.17	−0.14	0.35
Smoking within past 6 hours	−0.07	0.09	−0.009	0.18	−0.19	0.28
Prevalent CVD	0.07	0.05	−0.014	0.02	−0.36	0.02
Hypertension medication	0.14	0.0001	−0.019	0.0001	−0.53	0.0001
Lipid-lowering medication	0.07	0.01	−0.012	0.012	−0.33	0.007
Hormone replacement therapy	−0.11	0.001	0.006	0.30	0.26	0.06
Walk test done:						
Before brachial testing	−0.08	0.007	0.023	0.0001	0.65	0.0001
After brachial testing	−0.08	0.005	0.010	0.06	0.23	0.07

DBP indicates diastolic blood pressure; β, the adjusted regression coefficient for the covariate on the brachial measure (expressed per 1 SD for continuous covariates). Coefficients for walk test variables are referent to walk test not done.

brachial artery diameter, FMDmm, and FMD% from the following covariates, with $P \leq 0.05$ for inclusion: SBP, diastolic BP, heart rate, diabetes, cigarette smoking (current and within past 6 hours), total cholesterol/HDL cholesterol, triglycerides, body mass index (BMI), glucose, hypertension medication, lipid medication, hormone replacement therapy, and prevalent CVD.[15] Two variables coding the walk test (before versus not done and after versus not done) entered the models together or not at all. Heritability was estimated by variance-components methods (SOLAR).[16]

Results

Participant Characteristics and FMD

The characteristics of the 2883 study participants are listed in Table 1. The age range was 33 to 88 years. A total of 963 men and 1179 women underwent the 6-minute walk test, which preceded the brachial study in 51% of these participants. Mean FMD% was 3.3% (interquartile range, 1.1% to 4.9%) in women and 2.4% (interquartile range, 0.7% to 3.7%) in men (Table 2). Baseline brachial artery diameter was inversely correlated with FMDmm and FMD%. Figure 1 depicts that FMD declined with advancing age; FMDmm was similar in both sexes, whereas FMD% was higher in women than men until age 70 years.

Clinical Correlates of Brachial Diameter and FMD

In age- and sex-adjusted models, brachial artery measures were directly associated with most CVD risk factors and prevalent CVD (Table 3). The relation between FMD and CVD risk factors was confirmed in Figure 2, which displays that FMDmm and FMD% were significantly lower with advancing quintile of Framingham risk score.

The stepwise multivariable predictors of increasing FMD% were female gender, heart rate, and having the walk test first, whereas the predictors of decreasing FMD% were advancing age, SBP, BMI, lipid-lowering medication, and smoking within the past 6 hours (Table 4). Each 20-mm Hg increment in SBP was associated with a 0.62% reduction in FMD%. The relation of BP stage (JNCVI[17]) to brachial artery measures is displayed in Figure 3. The overall model explained 16% of the interindividual variability in FMD%; age, and sex explained ≈11%, SBP explained 4%, and each additional variable explained <1% of the variability. Multivariable correlates of FMDmm were similar to FMD%, except that female gender and BMI were not significantly associated.

Accounting for covariates retained in the stepwise models, the estimated heritability of the brachial artery baseline diameter was 0.33 (SE, 0.07), FMDmm was 0.13(SE, 0.06), and FMD% was 0.14 (SE, 0.06).

Confounding

We performed a series of analyses excluding various subject subsets, including those taking lipid medications, taking antihypertensive medications, undergoing hormone replacement therapy, with prevalent CVD, and ineligible for the walk test. In general, the multivariable models describing the factors associated with FMD were not substantively altered (data not shown).

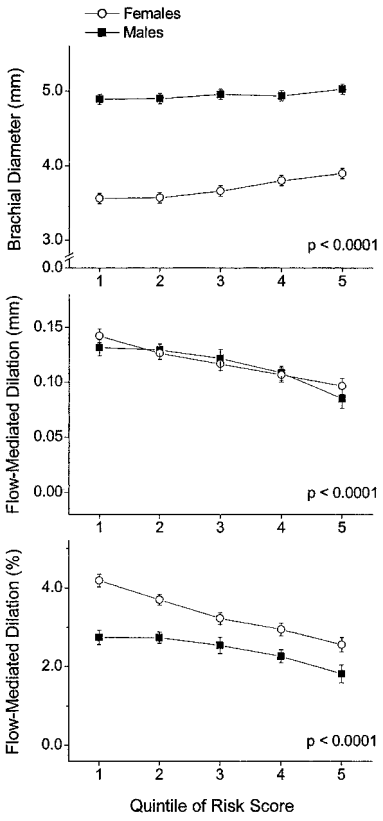

Figure 2. Mean and standard error of brachial measures by quintile of sex-specific Framingham risk score[38] (age not included in point score) in participants without prevalent CVD; *P* value is for age- and sex-adjusted trend test.

To determine the impact of the walk test on our results, we examined separate models for participants having the walk test before versus after brachial testing; the results were not substantively different (data not shown). In particular, the heart rate–FMD association was not secondary to exercise; the estimate for the effect of heart rate did not vary materially by walk-test timing.

Interactions

In sex-specific multivariable models, estimated effects of age on baseline brachial artery diameter and FMDmm were similar for both sexes. However, for FMD%, there was an age-gender interaction ($P=0.01$); the age effect was -0.5 for men and -0.7 for women, confirming steeper age-related decline of FMD% in women (Figure 1).

Discussion

Many prior studies have examined the determinants of endothelial function and reported dozens of clinical correlates, including numerous CVD risk factors. However, most previous studies were small and used case-control or case-series designs. Two studies were larger.[3,13] A study of 500 young adults (age, 36±15 years) found inverse relations between FMD% and smoking, older age, male gender, larger vessel size, and composite risk score,[3] but the generalizability of the study was limited by the exclusion of individuals with known hypertension and diabetes. Another study of 4040 mostly elderly individuals (75±13 years), derived from several databases, focused on technical issues related to studying FMD in population-based research.[13] The investigators reported lower FMD% with older age and male gender, but they did not examine CVD risk factors. The present study extends prior investigations by comprehensively examining the clinical correlates and heritability of endothelial function in a large middle-aged-to-elderly community-based cohort.

The strongest multivariable correlates of FMD in our study were age, gender, and SBP. Lower endothelial function with advancing age has been a consistent finding in prior studies.[13,18] The mechanisms remain uncertain but may relate to age-associated increases in reactive oxygen species production.[19] The significant age–gender interaction and steeper slope for the age-related decline in FMD% among women also is consistent with prior studies and has been attributed to

TABLE 4. Stepwise Linear Regression Models

	β	95% CI	P
Baseline diameter			
Sex, female vs male	−1.18	(−1.22 to −1.13)	<0.0001
Age, 10 y	0.09	(0.07 to 0.12)	<0.0001
BMI, 5 kg/m^2	0.15	(0.13 to 0.17)	<0.0001
Hypertension medication	0.07	(0.03 to 0.12)	0.002
Hormone replacement therapy	−0.09	(−0.15 to −0.03)	0.003
Model R^2=0.57			
FMD, mm			
Sex, female vs male	−0.003	(−0.011 to 0.004)	0.382
Age, 10 y	−0.020	(−0.025 to −0.016)	<0.0001
SBP, 20 mm Hg	−0.025	(−0.029 to −0.021)	<0.0001
Heart rate, 10 bpm	0.004	(0.001 to 0.008)	0.020
Walk test			
Before brachial testing	0.012	(0.002 to 0.022)	0.019
After brachial testing	0.001	(−0.009 to 0.011)	0.823
Lipid-lowering medication*	−0.011	(−0.020 to −0.001)	0.025
Smoked within 6 hours	−0.013	(−0.0264 to −0.0001)	0.0487
Model R^2 =0.12			
FMD, %			
Sex, female vs male	0.70	(0.51 to 0.89)	<0.0001
Age, 10 y	−0.58	(−0.69 to −0.47)	<0.0001
SBP, 20 mm Hg	−0.62	(−0.73 to −0.51)	<0.0001
BMI, 5 kg/m^2	−0.16	(−0.25 to −0.06)	0.001
Heart rate, 10 bpm	0.13	(0.04 to 0.22)	0.006
Walk test			
Before brachial testing	0.31	(0.05 to 0.56)	0.020
After brachial testing	−0.04	(−0.29 to 0.22)	0.779
Lipid-lowering medication*	−0.26	(−0.50 to −0.02)	0.035
Smoked within 6 hours	−0.34	(−0.67 to −0.01)	0.0439
Model R^2=0.16			

See legend to Table 3. There was a significant difference between the effects of walk test before vs after brachial artery testing for both the FMDmm ($\beta=0.011$, $P=0.01$) and the FMD% ($\beta=0.27$, $P=0.002$).

*Lipid-lowering medication was not randomized and may serve as a surrogate marker for preexisting hyperlipidemia; see Discussion section for indication bias.

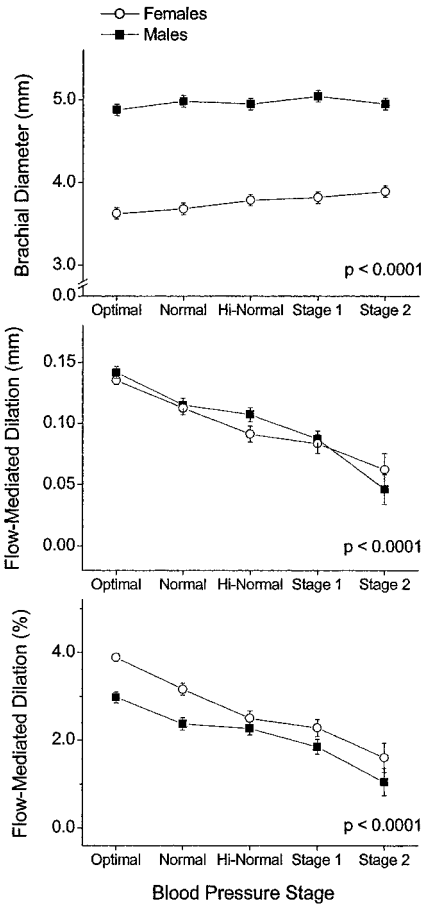

Figure 3. Mean and standard error of brachial measures by sex and Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure VI level[17] (optimal, normal, high normal, Stage I, Stage II) disregarding antihypertensive treatment; *P* value is for age- and sex-adjused trend test.

the postmenopausal state,[18] but it also may be explained partly by gender differences in baseline brachial artery size.

Our study provides evidence that SBP is an important correlate of endothelial dysfunction. Indeed, the gradient of diminished FMD begins at nonhypertensive BP levels (Figure 3). An association between hypertension and endothelial dysfunction has been observed in many smaller studies.[4,12] Research has demonstrated that antihypertensive treatment with some medications improves endothelial function,[20] and those patients with improved endothelial function have fewer incident CVD events.[8] The pathogenesis of the association between endothelial dysfunction and hypertension is not fully understood. Intriguing evidence suggests that endothelial dysfunction may antedate and possibly contribute to the development of essential hypertension.[21] However, the present cross-sectional study cannot determine whether endothelial dysfunction was a cause or consequence of hypertension or, alternatively, whether FMD and SBP were associated by virtue of their joint correlation with a third factor, such as arterial stiffness.

We found an inverse association between BMI and FMD%, which is consistent with earlier studies associating endothelial dysfunction with obesity,[6,22,23] which improved with weight loss.[22] The mechanisms are undoubtedly multifactorial but partially may be attributable to oxidative stress[23] and systemic inflammation.[22] However, we did not find that BMI was a multivariable predictor of FMD when expressed as FMDmm.

The examination's multistation design provided us with the opportunity to analyze the relation of exercise to FMD. We found that walk test before endothelial function testing was associated with enhanced FMD. Earlier studies have demonstrated that exercise training improves endothelial function,[24,25] but the acute effects of exercise on systemic endothelial function have been less well studied. Green et al[26] have reported NO-dependent increases in forearm blood flow during leg exercise, which could have improved brachial FMD in our participants.

An unexpected finding in our study was the observation that heart rate was positively associated with FMD. One prior investigation reported a negative correlation between heart rate and FMD%,[27] but that study involved only 29 men with metabolic syndrome. Our findings are compatible with animal studies demonstrating that higher-frequency pulsatile flow was associated with increased NO release.[28]

A genetic contribution to endothelial function has been supported by earlier studies demonstrating that young individuals with a family history of CVD have diminished endothelial function[29,30] and that genetic polymorphisms may influence endothelial function.[31,32] Heritability estimates the portion of the variability accounted for by genetic factors after adjusting for the clinical correlates retained in the model. Our heritability estimate of 0.12 to 0.14 for FMD suggests that genetic factors contribute modestly to the variability in endothelial function.

We observed that smoking within 6 hours before testing was associated with impaired FMD. The deleterious impact of passive[33] and chronic active[3] cigarette smoking on endothelial function has been well described and may be one of the mechanisms contributing to increased risk of CVD events in exposed individuals.

Several of our findings differed from previous reports. For example, the apparently paradoxical finding of an inverse association between FMD and lipid-lowering medications should not be construed to mean that lipid lowering caused lower FMD. Rather, the cross-sectional, observational data likely reflect confounding by indication bias, whereby individuals with higher lipid levels, a higher burden of CVD risk factors, and prevalent CVD were prescribed treatment with medication.

In addition, serum cholesterol,[5] impaired glucose tolerance,[34] and diabetes[5] have been inversely associated with FMD in prior studies. Whereas total/HDL cholesterol, glucose level, and diabetes were associated with impaired FMD in age- and sex-adjusted models, they were not retained in the multivariable models. There are several plausible explanations for the apparent discrepancies with prior research. The lack of independence of the variables could be attributable to the clustering of hypertension, glucose intolerance, lipid abnormalities, and obesity in individuals with the metabolic syndrome, resulting in colinearity among these variables. Our

study may have been better able to adjust for potential confounders because of the large sample size and community-based design. Alternatively, it may have been difficult to detect these relations because we studied middle-aged-to-elderly individuals with a typical distribution of lipid and glucose values and relatively low mean FMD%. It has been suggested that when there are multiple competing risk factors, the effects of specific risk factors may be overwhelmed by the accumulated effects of others.[35] Nevertheless, we cannot exclude the possibility that more sophisticated measures of impaired glucose tolerance may be related to FMD.

Baseline brachial diameter was strongly associated with CVD risk factors. Holubkov et al[36] reported a similar association between baseline diameter and both risk factors and prevalent coronary artery disease in women, although this relation is not generally appreciated. Baseline diameter also had higher heritability than FMDmm or FMD% after controlling for body size and risk factors. The mechanisms for these observations are unknown and merit additional investigation.

Limitations and Strengths

This study has several limitations. Because the study was performed with community-based volunteers, it would have been inappropriate to withhold CVD medications. A recent study, however, suggested that administration of vasoactive medications not containing nitrates does not significantly influence FMD% acutely,[37] and secondary analyses excluding participants taking lipid-lowering or antihypertensive medications did not substantively alter the results. It also was unfeasible to administer nitroglycerin to our community-based volunteers; thus, we do not have a measure of non–endothelium-dependent vasodilation. Although we cannot exclude the possibility that alterations in vascular smooth muscle function influenced our results, FMD is well accepted as a noninvasive measure of endothelial function.[1] Finally, our cohort was overwhelmingly white, so generalizability to other ethnicities is unknown. A prior study, however, suggests that FMD% is comparable in black and white populations.[4] Notwithstanding the above limitations, the present study has several strengths, including the large sample of middle-aged and elderly adults, resulting in excellent power to describe the multivariable correlates of FMD, the community-based cohort with routine measurements of covariates minimizing bias, and the rigorous quality-control protocol, enhancing the quality of the measurements.

Clinical Implications

Our study suggests that endothelial function, as measured by FMD, has both environmental and genetic determinants. Decreased FMD% was associated with CVD risk factors, particularly advancing age, higher SBP, BMI, and recent cigarette smoking. The apparent acute effects of exercise on endothelial function provide insight into potential vascular benefits of exercise. In addition, FMD was modestly heritable, but the precise genetic determinants of endothelial function will require additional study. The finding that a substantial portion of the variability of FMD remains unexplained by genetic and standard CVD risk factors indicates that the relation of novel risk factors to FMD also merits additional investigation.

Overall, our results are consistent with the growing evidence that risk factors contribute to the development of atherosclerosis in part by impairing endothelial function. Longitudinal follow-up of this and other cohorts will be required to determine whether endothelial dysfunction predicts outcome in the community independent of associated CVD risk factors.

Acknowledgments

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