Cross-Sectional Relations of Peripheral Microvascular Function, Cardiovascular Disease Risk Factors, and Aortic Stiffness

The Framingham Heart Study

Gary F. Mitchell, MD; Joseph A. Vita, MD; Martin G. Larson, SD; Helen Parise, ScD; Michelle J. Keyes, MA; Elaine Warner, BS; Ramachandran S. Vasan, MD; Daniel Levy, MD; Emelia J. Benjamin, MD, ScM

Background—Aortic stiffness and small-artery structure and function share various risk factors; however, relations between these 2 measures of vascular function are complex and incompletely understood.

Methods and Results—We examined hyperemic forearm blood flow, an indicator of microvascular structure and function, and aortic stiffness in 2045 participants (1107 women, mean age 61±9 years) in the Framingham Heart Study offspring cohort. Using arterial tonometry, we evaluated 3 measures of aortic stiffness: brachial pulse pressure; carotid-femoral pulse wave velocity (CFPWV), which is related directly to aortic wall stiffness; and forward pressure wave amplitude (P_f), which is related directly to aortic wall stiffness and inversely to aortic diameter. Using high-resolution ultrasound and Doppler, we evaluated brachial artery diameter, blood flow, and forearm vascular resistance (FVR) at baseline and during reactive hyperemia after 5 minutes of forearm ischemia. In multivariable models that adjusted for cardiovascular disease risk factors, local brachial pulse pressure, CFPWV, and P_f, considered separately, were associated with increased baseline and hyperemic FVR ($P<0.001$). In models that further adjusted for mean arterial pressure, each measure of aortic stiffness was associated with reduced hyperemic flow ($P<0.001$). In risk factor–adjusted models that simultaneously considered CFPWV and P_f, both were associated with increased FVR at baseline ($P<0.01$) and during hyperemia ($P<0.001$).

Conclusions—Our findings indicate that abnormal aortic stiffness and increased pressure pulsatility are associated with blunted microvascular reactivity to ischemic stress that is in excess of changes attributable to conventional cardiovascular disease risk factors alone, including mean arterial pressure. **(*Circulation*. 2005;112:3722-3728.)**

Key Words: endothelium ■ microcirculation ■ risk factors ■ epidemiology

Aortic stiffness increases markedly with advancing age[1] and is associated with elevated systolic and pulse pressure. Aortic stiffness, as indicated by increased pulse pressure, is associated with a variety of common afflictions of aging, including cardiovascular disease,[2–4] stroke,[5,6] cognitive disorders,[7–9] white matter lesions,[10,11] macular degeneration,[12] renal dysfunction,[13,14] osteoporosis,[15] and glucose intolerance.[16–18] Microvascular dysfunction appears to represent a common element in the pathophysiology of these diverse conditions.[12,13,19–22] Several studies have suggested that increased aortic stiffness and elevated pulse pressure may stimulate hypertrophy, remodeling, or rarefaction in the microcirculation, leading to increased resistance to mean flow.[23–26] Endothelial function in medium-sized and smaller arteries may also be impaired by increased pressure pulsatility.[27,28] Because structure and function in small arteries may be adversely affected by exposure to high pulsatile pressure, the possibility exists that increased aortic stiffness and elevated pulse pressure may contribute directly to the pathogenesis of the heterogeneous group of small-vessel disorders and diseases described above.

We have previously shown that microvascular reactivity, as assessed by hyperemic flow after forearm cuff occlusion, is related to standard cardiovascular disease risk factors.[29] However, to the best of our knowledge, no prior study has reported the relations between direct measures of aortic stiffness and peripheral microvascular function in a large community-based sample. Therefore, we assessed aortic stiffness using tonometry and forearm microvascular function by evaluating reactive hyperemia in response to forearm cuff occlusion in participants in the Framingham Heart Study. We hypothesized that vascular risk factors would be associated

Received March 22, 2005; revision received August 23, 2005; accepted September 12, 2005.

From Cardiovascular Engineering, Inc (G.F.M., E.W.), Waltham, Mass; Department of Mathematics and Statistics (M.G.L., H.P.), Evans Department of Medicine (J.A.V., R.S.V., E.J.B.), Whitaker Cardiovascular Institute (J.A.V., R.S.V., E.J.B.), and Section of Preventive Medicine (R.S.V., D.L., E.J.B.), Boston University School of Medicine, Boston, Mass; the National Heart, Lung, and Blood Institute (D.L.), Bethesda, Md; and the NHLBI's Framingham Study (M.G.L., M.J.K., R.S.V., D.L., E.J.B.), Framingham, Mass.

Guest Editor for this article was Mark A. Creager, MD.

Correspondence to Gary F. Mitchell, MD, Cardiovascular Engineering, Inc, University Office Park, Bldg 2, 51 Sawyer Rd, Suite 100, Waltham, MA 02453. E-mail GaryFMitchell@mindspring.com

Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/CIRCULATIONAHA.105.551168**

with alterations in forearm microvascular function at rest and in response to a hyperemic stimulus and that aortic stiffness measures would have an incremental relation with blunted hyperemic vasodilatory reserve after adjustment for other clinical correlates of vascular dysfunction.

Methods

Study Participants

The design of the Framingham Offspring Study has been described.[30] Participants in the seventh examination cycle (1998–2001) were eligible for this investigation (n=3539). Analyses were performed in 2045 subjects with both tonometry and brachial artery test data. Details of the participant tonometry and brachial evaluations, data acquisition, and data analysis have been presented previously.[29] The examination included a 6-minute walk test (Bruce protocol stages I and II) in participants without contraindications (known coronary heart disease, chest pain on test day, or inability to perform test). The Boston Medical Center Institutional Review Board approved the protocol, and all participants gave written informed consent.

Image and Flow Analyses

Brachial artery diameter was measured as described previously.[31] Flows were analyzed from the digitized Doppler audio data with a semiautomated signal-averaging approach as detailed previously.[29] After cuff deflation, sonographers monitored and recorded flow (for up to 15 seconds after cuff release) until flow peaked, which generally occurred within 3 to 5 beats. During subsequent analysis by a randomly assigned sonographer, timing of peak flow was visually confirmed from a raw spectral analysis of individual beats; only beats representing the peak flow response (generally 2 to 4 beats) were marked for inclusion in the signal-averaged spectrum. Flow spectra were signal-averaged (1000 Hz resolution) with the ECG as a fiducial point. Baseline and hyperemic flow velocities were assessed directly and were multiplied by baseline brachial artery cross-sectional area to obtain volume flows, which are needed to compute forearm vascular resistance (FVR). FVR was calculated by dividing mean arterial pressure (MAP), which was assumed constant throughout the test, by mean volume flow at baseline and during hyperemia.

Measures of Aortic Stiffness

We evaluated 3 related measures of aortic stiffness: brachial pulse pressure, central forward pressure wave amplitude (P_f), and carotid-femoral pulse wave velocity (CFPWV). Brachial pulse pressure was examined as a measure of the pulsatility of pressure just proximal to the vascular bed of interest; however, brachial pulse pressure is affected by local reflections, which in turn are influenced by local resistance. Thus, it is possible that abnormal resistance vessel function and increased local wave reflection could produce apparent relations between local pulse pressure and vascular resistance that do not involve aortic stiffness. We therefore examined P_f from the carotid pressure waveform as a measure of proximal aortic impedance to pulsatile flow that is minimally affected by wave reflections. P_f is closely related to characteristic impedance of the aorta, which is affected by wall stiffness and thickness and is especially sensitive to matching between pulsatile flow and aortic diameter.[32] We also evaluated CFPWV as an indicator of aortic wall stiffness that is less dependent on aortic diameter.[32]

Measures of Microvascular Function

We examined baseline FVR, which depends on microvascular density, tone, and structure.[33] We also measured FVR during reactive hyperemia after 5 minutes of forearm cuff occlusion, which reflects the near maximal dilation of forearm microvessels in response to local ischemia and local shear stress. Aortic stiffness measures, such as CFPWV, are dependent on distending pressure.[1] Because FVR includes MAP in the calculation, any observed relation between aortic stiffness and FVR may be attributable to the common MAP term. Therefore, as alternative measures of microvascular function, we evaluated mean brachial flow velocity at baseline and during reactive hyperemia and included a separate adjustment for MAP in these multivariable flow models. We used flow velocity because it is less sensitive to body size than volume flow.

TABLE 1. Tonometry Data and Brachial Artery Hemodynamics

Variable	Baseline	Hyperemia
Brachial pulse pressure, mm Hg	53±15	...
Forward pressure wave, mm Hg	41±13	...
MAP, mm Hg	92±12	...
CFPWV, m/s	10.0±3.4	...
Mean flow velocity, cm/s	8.0±4.9	51.1±21.3
Mean volume flow, mL/s	1.2±0.9	7.2±3.7
FVR, ln (dyne · s^{-1} · cm^{-5})	11.8±0.7	9.9±0.6

Statistical Analysis

Regression was performed with the REG procedure in SAS version 8.0.[34,35] Values for FVR were highly skewed and were therefore natural log-transformed to normalize the distribution. We evaluated multivariable cross-sectional relations of various cardiovascular disease risk factors (see below) with brachial flow velocity and FVR using stepwise multivariable regression models that adjusted for age and sex in all models and also for MAP in flow models. These models did not consider stiffness measures. Subsequently, we used multivariable regression to determine whether aortic stiffness variables were related to microvascular function variables (FVR and flow velocity [dependent variables]) after adjusting for other known or potential correlates of FVR. Additional covariates were selected on the basis of prior publications and included MAP (for flow models only), heart rate, body mass index, fasting glucose, total/HDL cholesterol ratio, triglycerides, prevalent cardiovascular disease, diabetes, current cigarette smoking, smoking within 6 hours of the evaluation, antihypertensive treatment, lipid-lowering treatment, daily aspirin use, hormone replacement therapy, and timing of walk test.[1,29,31] Walk test performance (modeled as "before" versus "after" vascular testing versus "not done," with the latter as referent) was incorporated into multivariable models. A 2-sided $P<0.05$ was considered statistically significant.

Results

Characteristics of the study participants have been reported previously.[29] Briefly, the 1107 women and 938 men in the sample were middle-aged to elderly, with a mean age of 61±9 years (range 33 to 87 years) at examination. Prevalences of cardiovascular disease (12%), diabetes (12%), hypertension (44%), use of antihypertensive (32%) or lipid-lowering (20%) medications, daily aspirin use (29%), and smoking within the prior 12 months (14%) or 6 hours (9%) were typical for a community-based cohort of this age. Hormone replacement therapy was used by 37% of the women. A walk test was performed by 76% of participants either before (39%) or after (37%) brachial testing. As previously reported, baseline brachial artery diameter was 3.7±0.6 mm in women and 4.9±0.6 mm in men.[29] Aortic stiffness measures at baseline and brachial artery hemodynamics at baseline and during reactive hyperemia are presented in Table 1. On average, brachial artery mean flow increased substantially (≈6-fold) during reactive hyperemia (Table 1).

TABLE 2. Multivariable Relations Between Common Covariates and FVR

	FVR, ln(dyne · s^{-1} · cm^{-5})					
	Baseline			Hyperemia		
Variable	Estimate	SE	*P*	Estimate	SE	*P*
Age (9 y)	0.09	0.01	<0.001	0.15	0.01	<0.001
Female (vs male)	0.62	0.03	<0.001	0.41	0.02	<0.001
Heart rate (11 bpm)	−0.07	0.01	<0.001	...	...	...
Body mass index (4.6 kg/m^2)	−0.17	0.01	<0.001	−0.04	0.01	0.008
Fasting glucose (26 mg/dL)	...	...	...	0.02	0.01	0.021
Total/HDL cholesterol (1.3)	−0.04	0.02	0.008	...	...	...
Prevalent cardiovascular disease	...	...	...	0.06	0.04	0.089
Smoking	−0.22	0.04	<0.001	...	...	...
Antihypertensive medication	...	...	...	0.11	0.03	<0.001
Hormone replacement therapy	−0.06	0.04	0.097	...	...	...

All continuous variables expressed per 1 SD; categorical variables expressed as present vs absent. Baseline model R^2=0.31, hyperemia model R^2=0.23.

Cross-Sectional Correlates of FVR and Flow Velocity at Baseline and During Hyperemia

Multivariable cross-sectional correlates of baseline and hyperemic FVR and brachial flow velocity are presented in Tables 2 and 3. Increasing age was associated with lower baseline and hyperemic flow and higher FVR. Women had lower flow and higher FVR at baseline than men. Women had a greater increase in flow than men during hyperemia, which eliminated the difference in brachial flow and reduced the difference in FVR between men and women, although hyperemic FVR remained higher in women. Hormone replacement therapy was associated with higher flows at baseline and during hyperemia but was not significantly related to FVR.

Several cardiovascular disease risk factors, including higher heart rate, increasing body mass index, elevated fasting glucose, higher total/HDL cholesterol ratio, and smoking, were associated with elevated resting flow (Table 3) and lower FVR (Table 2) at baseline. In contrast, risk factors were generally associated with a blunted flow response and higher FVR during hyperemia. For example, fasting glucose tended to be associated with higher flow during baseline but was associated with reduced flow and increased FVR during hyperemia. Increasing body mass index was associated with higher flow at baseline but was unrelated to flow during hyperemia, which suggests that flow reserve was blunted. Prevalent cardiovascular disease and treatment with antihypertensive medication were associated with reduced flow during hyperemia (Table 3).

Cross-Sectional Relations of Aortic Stiffness Measures, FVR, and Flow Velocity at Baseline and During Hyperemia

Relations between aortic stiffness measures and FVR are presented in Table 4. In age- and sex-adjusted models, higher

TABLE 3. Multivariable Relations Between Common Covariates and Brachial Flow Velocity

	Brachial Flow Velocity, cm/s					
	Baseline			Hyperemia		
Variable	Estimate	SE	*P*	Estimate	SE	*P*
Age (9 y)	−0.82	0.11	<0.001	−8.18	0.43	<0.001
Female (vs male)	−1.21	0.25	<0.001	0.95	0.94	0.32
MAP (12 mm Hg)	−0.09	0.11	0.41	−5.33	0.43	<0.001
Heart rate (11 bpm)	0.77	0.11	<0.001	1.73	0.41	<0.001
Body mass index (4.6 kg/m^2)	0.75	0.11	<0.001	...	...	...
Fasting glucose (26 mg/dL)	0.21	0.11	0.054	−0.95	0.42	0.024
Total/HDL cholesterol (1.3)	0.39	0.11	0.001	...	...	...
Prevalent cardiovascular disease	−0.95	0.33	0.004	−4.06	1.30	0.002
Smoking	2.10	0.30	<0.001	...	...	...
Antihypertensive medication	...	...	...	−2.92	0.92	0.002
Hormone replacement therapy	0.70	0.29	0.02	2.22	1.12	0.05

All continuous variables expressed per 1 SD; categorical variables expressed as present vs absent. Baseline model R^2=0.16, hyperemia model R^2=0.31.

TABLE 4. Relations Between Measures of Arterial Stiffness and Microvascular Function

	FVR, ln(dyne · s^{-1} · cm^{-5})					
	Baseline			Hyperemia		
Variable and Model	Estimate	SE	*P*	Estimate	SE	*P*
Individual variables						
Brachial pulse pressure						
Age-sex model	0.062	0.016	<0.001	0.170	0.012	<0.001
Multivariable model*	0.086	0.016	<0.001	0.175	0.012	<0.001
Forward pressure wave						
Age-sex model	0.038	0.015	0.014	0.159	0.011	<0.001
Multivariable model*	0.071	0.015	<0.001	0.165	0.012	<0.001
CFPWV						
Age-sex model	−0.001	0.018	0.95	0.132	0.013	<0.001
Multivariable model*	0.072	0.018	<0.001	0.139	0.014	<0.001
Joint models						
Age-sex model						
Forward pressure wave	0.045	0.017	0.007	0.134	0.012	<0.001
CFPWV	−0.019	0.019	0.32	0.080	0.014	<0.001
Multivariable model*						
Forward pressure wave	0.057	0.016	<0.001	0.142	0.012	<0.001
CFPWV	0.051	0.019	0.007	0.086	0.015	<0.001

*Adjusted for age, sex, body mass index, heart rate, total/HDL cholesterol ratio, triglycerides, fasting glucose, prevalent cardiovascular disease, diabetes, need for lipid-lowering or antihypertensive therapy, current smoking, smoking 6 hours before test, hormone replacement (in women), timing of walk test (not done, before, after), and daily aspirin use.

All continuous variables expressed per 1 SD.

TABLE 5. Relations Between Measures of Arterial Stiffness and Microvascular Function

	Brachial Flow Velocity, cm/s					
	Baseline			Hyperemia		
Variable and Model	Estimate	SE	*P*	Estimate	SE	*P*
Individual variables						
Brachial pulse pressure						
Age-sex-MAP model	−0.09	0.13	0.50	−2.16	0.50	<0.001
Multivariable model *	−0.02	0.13	0.91	−1.93	0.51	<0.001
Forward pressure wave						
Age-sex-MAP model	0.15	0.13	0.25	−1.86	0.47	<0.001
Multivariable model *	0.13	0.12	0.29	−1.69	0.48	<0.001
CFPWV						
Age-sex-MAP model	0.08	0.14	0.57	−2.22	0.54	<0.001
Multivariable model *	−0.36	0.15	0.014	−2.27	0.57	<0.001
Joint models						
Age-sex-MAP model						
Forward pressure wave	0.13	0.13	0.30	−1.50	0.48	0.002
CFPWV	0.05	0.15	0.74	−1.86	0.55	<0.001
Multivariable model *						
Forward pressure wave	0.21	0.13	0.10	−1.34	0.49	0.007
CFPWV	−0.41	0.15	0.006	−1.95	0.58	<0.001

*See Table 4 legend for covariates included in multivariable models, plus MAP.

All continuous variables expressed per 1 SD.

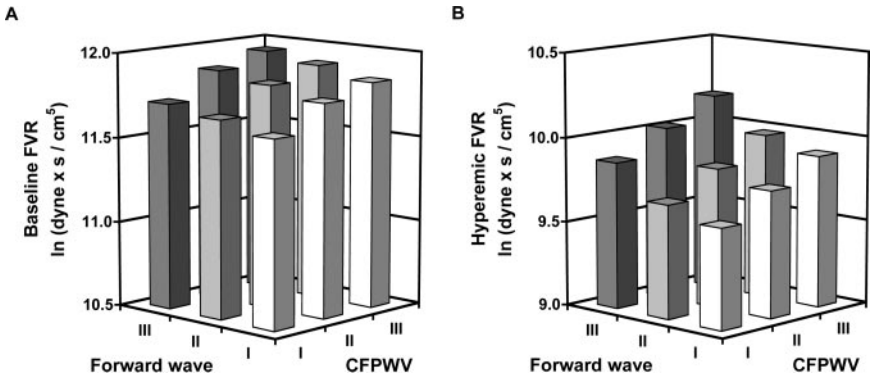
brachial pulse pressure and P_f were modestly associated with increased baseline FVR, whereas CFPWV was not related to baseline FVR when evaluated alone or together with P_f in the same model. Relations between baseline FVR and aortic stiffness measures persisted (and CFPWV became significant) in multivariable models that included cardiovascular disease risk factors and other potentially confounding covariates (Table 4). Aortic stiffness measures were related to hyperemic FVR in age- and sex-adjusted and multivariable models (Table 4). In these models, partial correlations attributable to the stiffness variables (partial R^2=0.11 to 0.17) accounted for approximately half of the variance explained by the model (model R^2=0.27 to 0.31). CFPWV and P_f were related to higher hyperemic FVR whether considered individually or jointly. Similar models revealed minimal relations between aortic stiffness measures and baseline flow velocity (Table 5). However, stiffness measures were associated with blunted hyperemic flow response (Table 5).

Multivariable-adjusted baseline and hyperemic FVR according to tertiles of P_f and CFPWV are presented in the Figure. Baseline FVR increased modestly with increasing tertiles of CFPWV (P<0.001) or P_f (P=0.003; Figure, panel A). Hyperemic FVR was substantially higher with increasing tertiles of CFPWV (P<0.001) or P_f (P<0.001; Figure, panel B). As a result, hyperemic FVR was >2-fold higher in individuals who fell within the highest as compared with the lowest tertiles for both CFPWV and P_f.

Discussion

Our community-based study evaluated cross-sectional relations between measures of aortic stiffness and peripheral microvascular structure and function and demonstrated that increased aortic stiffness was associated with higher FVR at baseline and during reactive hyperemia and with blunted flow reserve during hyperemia. Both microvascular reactivity[22,25,36–38] and aortic stiffness[1,16–18] are related to various cardiovascular disease risk factors, including advancing age, hypertension, diabetes, hypercholesterolemia, and obesity. Hence, the association between microvascular function and aortic stiffness may be partially attributable to parallel effects of shared risk factors. However, in the present analyses, relations between increased aortic stiffness and impaired microvascular structure or function, as assessed by hyperemic response to ischemia, persisted in models that adjusted for conventional risk factors, which indicates that aortic stiffening is accompanied by microcirculatory structural or functional remodeling beyond that which is explained by contemporaneously measured cardiovascular disease risk factors.

Certain measures of aortic stiffness, such as CFPWV, increase with distending pressure (MAP) because of the nonlinear stress-strain relations of the arterial wall.[39] Because MAP is closely related to FVR, relations between aortic stiffness and FVR may be partially attributable to elevated MAP. However, we found relations between increased aortic stiffness and reduced hyperemic flow that persisted even after adjusting for MAP and other risk factors. Thus, the relation between aortic stiffness and microvascular function, when



Relations between P_f, CFPWV, and FVR measured at baseline (A) and during reactive hyperemia (B). A, At baseline, increasing levels of P_f or CFPWV were associated with increased FVR. B, These associations were accentuated during hyperemia, with steep, graded relations across the spectrum from lowest tertiles of P_f or CFPWV to highest tertiles for each variable. Cutpoints for tertiles occurred at 8.2 and 10.3 m/s for CFPWV and at 34 and 44 mm Hg for P_f. Data are least square means of natural log (ln) transformed FVR, adjusted for age, sex, body mass index, heart rate, total/HDL cholesterol ratio, triglycerides, fasting glucose, prevalent cardiovascular disease, diabetes, use of lipid-lowering or antihypertensive therapy, smoking, hormone replacement in women, timing of walk test, and daily aspirin use.

assessed in terms of hyperemic flow reserve, is not fully explained by passive effects of distending pressure on aortic stiffness.

If changes in the relation between MAP and flow (ie, FVR) involve microvascular structural remodeling or rarefaction, as opposed to a reversible change in microvascular tone, impaired responsiveness to metabolic demand may result. Our finding of blunted hyperemic flow in individuals with increased aortic stiffness despite a relatively severe metabolic stimulus (5 minutes of ischemia) suggests that factors contributing to higher baseline FVR are not immediately reversible and may involve a structural component. Consistent with this hypothesis, prior studies in animal models have shown that locally induced isolated alterations in pressure pulsatility have major effects on microvascular structure and function.[23,24,26,40,41] Similar relations between higher 24-hour pulse pressure and increased microvascular media-lumen ratio have been reported in individuals with hypertension.[25] These prior studies and our present results provide support for important relations between aortic stiffness, pressure pulsatility, and microvascular structure.

As in prior studies, we found that elevated fasting glucose, prevalent cardiovascular disease, and hypertension were associated with impaired vasodilatory reserve, as indicated by reduced flow and elevated FVR during hyperemia.[22,42,43] However, we also observed relations between several risk factors and elevated baseline forearm blood flow. In multivariable analyses, higher body mass index, an increased total/HDL cholesterol ratio, and active smoking were associated with elevated flow and reduced FVR at baseline. A trend toward higher baseline flow with increasing fasting glucose was also present. A similar pattern of increased resting forearm blood flow has been observed in response to acute hypertriglyceridemia.[44] Recent basic studies have shown that endothelial nitric oxide synthase is activated in the presence of oxidative stress.[45] Because oxidative stress may be increased in association with many of the risk factors noted above, the resulting increase in basal production of nitric oxide may have contributed to the observed increase in basal flow.

Our finding of elevated baseline flow and reduced FVR in smokers has not been described previously and seems contradictory to the findings of numerous prior studies that have shown a sympathetically mediated vasoconstrictor response to acute tobacco smoke or nicotine.[46–48] Recent studies have shown that nicotine may also have an angiogenic effect,[49–55] which could reduce resting microvascular resistance despite the vasoconstrictor response to acute nicotine exposure. Nicotinic acetylcholine receptors are expressed in various nonneuronal cells, including endothelial cells and vascular smooth muscle cells.[51] In vitro and in vivo models have shown that nicotine promotes endothelial cell proliferation and neovascularization, which are inhibited by selective nicotinic acetylcholine receptor antagonism.[49–52,54] Nicotine may also increase levels or enhance the activity of various growth factors, including vascular endothelial growth factor and platelet-derived growth factor.[53,54] Furthermore, neovascularization in response to nicotine may be enhanced with advancing age,[54] which may have contributed to the relatively large effect of smoking on baseline forearm blood flow in the elderly sample in the present study. In addition, there are many other constituents and potential mechanisms of vascular damage related to cigarette smoke, as recently reviewed.[56]

The presence of reduced baseline microvascular resistance and elevated flow in the presence of obesity or elevated total/HDL cholesterol ratio and in smokers may have deleterious effects on vascular reactivity and end-organ function that are amplified in the setting of increased arterial stiffness and elevated pulse pressure. Reduced regional vascular resistance can diminish the pressure drop in the precapillary arterioles and expose the capillaries to potentially harmful levels of pressure pulsatility.[40] This form of microvascular hypertension has been documented in people with diabetes mellitus and is thought to play an important role in the progression of diabetic small-vessel disease.[57,58] Elevated body mass index or total/HDL cholesterol ratio and active smoking may also damage the microcirculation through similar effects on precapillary resistance vessel structure or function, ultimately leading to tissue injury and impaired flow reserve despite elevated resting flow. Cigarette smoking is the leading risk factor for senile macular degeneration, especially the form of this disease that is associated with neovascularization.[54] Smoking is also a major risk factor for microalbuminuria and renal disease, which may involve an early period of hyperfiltration similar to that described for diabetic nephropathy.[59] Thus, microvascular damage may result from

increased aortic stiffness and elevated forward wave amplitude or from increased transmission of a given forward wave into the microcirculation because of reduced microvascular resistance. The combination of increased aortic stiffness and reduced resting microvascular resistance may be especially deleterious to the microcirculation of the eye, kidney, brain, and other organs.

Several limitations of the present study must be acknowledged. Because our study was cross-sectional, the directionality of the observed associations between aortic stiffness measures and microvascular function cannot be ascertained. Whereas we examined the relation of stiffness measures to baseline and hyperemic forearm blood flow and FVR (dependent variables), we cannot exclude the possibility that abnormalities in peripheral blood flow and FVR led to perturbations in aortic stiffness. We speculate but cannot prove in our cross-sectional observational study that the effects are bidirectional. We did not measure forearm volume, which affects FVR and may therefore affect the relation between FVR and arterial stiffness. To address this limitation, we included body mass index in the multivariable models and also performed analyses using flow velocity as the dependent variable instead of FVR, because flow velocity is less sensitive to body size. We used baseline diameter to compute early postdeflation volume flow. Diameter may decrease during the occlusion period because of subnormal flow levels. Furthermore, the sudden increase in flow after cuff release may further reduce diameter because of the Bernoulli phenomenon. Countering these effects is the potential for early dilation within the first 15 seconds after cuff release. The balance of these effects may have resulted in a slight underestimation or overestimation of volume flow in individual cases.

In addition, the cohort was middle-aged to elderly and largely white. Hence, the generalizability of our findings to other racial or ethnic groups or younger individuals is unknown. Because the cohort is community-based, it was inappropriate to withhold medications, which may have influenced our results. In relating 2 measures of microvascular tone to 3 measures of aortic stiffness and various cardiovascular disease risk factors, we have performed multiple statistical comparisons. However, the dependent variables and stiffness measures were closely correlated, and associations between hyperemic flow or FVR and stiffness measures were consistent and highly significant, which makes a spurious association less likely.

In summary, in our cross-sectional, community-based study, increased aortic stiffness and elevated arterial pressure pulsatility were associated with abnormalities in microvascular function as assessed by alterations in baseline and hyperemic FVR and flow. Various cardiovascular disease risk factors were related to impaired microvascular vasodilatory reserve, possibly owing to effects on large-artery properties. However, these common risk factors did not fully explain the relations between aortic stiffness and impaired microvascular response to ischemia. Thus, known and novel environmental and genetic influences, including conventional cardiovascular disease risk factors, may impact microvascular function through effects on aortic stiffness and pulsatile load, providing a possible explanation for associations between aortic stiffness and an emerging spectrum of disorders that share a microvascular etiology.

Acknowledgments

The Framingham Heart Study is funded by National Institutes of Health contract N01-HC-25195. This work was funded in part by grants R01-HL70100 and R01-HL60040 and by the Donald W. Reynolds Foundation. Dr Vasan was supported in part by grant K24-HL-04334.

Disclosure

Dr Mitchell is owner of Cardiovascular Engineering, Inc, a company that designs and manufactures devices that measure vascular stiffness. The company uses these devices in clinical trials that evaluate the effects of diseases and interventions on vascular stiffness. No other author has any ownership rights or other financial relationship with Cardiovascular Engineering, Inc. Dr Levy has served as a consultant to and/or lecturer for Pfizer, Bristol-Myers Squibb, GlaxoSmithKline, and Merck; these consultancies were terminated in 2003 or earlier. The other authors report no conflicts.

References

1. Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, Vasan RS, Levy D. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. *Hypertension*. 2004;43:1239–1245.
2. Mitchell GF, Moye LA, Braunwald E, Rouleau JL, Bernstein V, Geltman EM, Flaker GC, Pfeffer MA, for the SAVE investigators: Survival and Ventricular Enlargement Study. Sphygmomanometrically determined pulse pressure is a powerful independent predictor of recurrent events after myocardial infarction in patients with impaired left ventricular function. *Circulation*. 1997;96:4254–4260.
3. Chae CU, Pfeffer MA, Glynn RJ, Mitchell GF, Taylor JO, Hennekens CH. Increased pulse pressure and risk of heart failure in the elderly. *JAMA*. 1999;281:634–639.
4. Franklin SS, Khan SA, Wong ND, Larson MG, Levy D. Is pulse pressure useful in predicting risk for coronary heart disease? The Framingham Heart Study. *Circulation*. 1999;100:354–360.
5. Laurent S, Katsahian S, Fassot C, Tropeano AI, Gautier I, Laloux B, Boutouyrie P. Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. *Stroke*. 2003;34:1203–1206.
6. Domanski MJ, Davis BR, Pfeffer MA, Kastantin M, Mitchell GF. Isolated systolic hypertension: prognostic information provided by pulse pressure. *Hypertension*. 1999;34:375–380.
7. Launer LJ, Masaki K, Petrovitch H, Foley D, Havlik RJ. The association between midlife blood pressure levels and late-life cognitive function: the Honolulu-Asia Aging Study. *JAMA*. 1995;274:1846–1851.
8. Launer LJ, Ross GW, Petrovitch H, Masaki K, Foley D, White LR, Havlik RJ. Midlife blood pressure and dementia: the Honolulu-Asia aging study. *Neurobiol Aging*. 2000;21:49–55.
9. Kivipelto M, Helkala EL, Laakso MP, Hanninen T, Hallikainen M, Alhainen K, Iivonen S, Mannermaa A, Tuomilehto J, Nissinen A, Soininen H. Apolipoprotein E epsilon4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. *Ann Intern Med*. 2002;137:149–155.
10. Liao D, Cooper L, Cai J, Toole J, Bryan N, Burke G, Shahar E, Nieto J, Mosley T, Heiss G. The prevalence and severity of white matter lesions, their relationship with age, ethnicity, gender, and cardiovascular disease risk factors: the ARIC Study. *Neuroepidemiology*. 1997;16:149–162.
11. Duprez DA, De Buyzere ML, Van den NN, Simoens J, Achten E, Clement DL, Afschrift M, Cohn JN. Relationship between periventricular or deep white matter lesions and arterial elasticity indices in very old people. *Age Ageing*. 2001;30:325–330.
12. Klein R, Klein BE, Tomany SC, Cruickshanks KJ. The association of cardiovascular disease with the long-term incidence of age-related maculopathy: the Beaver Dam eye study. *Ophthalmology*. 2003;110:636–643.
13. Safar ME, London GM, Plante GE. Arterial stiffness and kidney function. *Hypertension*. 2004;43:163–168.
14. Mitchell GF. Increased aortic stiffness: an unfavorable cardiorenal connection. *Hypertension*. 2004;43:151–153.

15. Hirose K, Tomiyama H, Okazaki R, Arai T, Koji Y, Zaydun G, Hori S, Yamashina A. Increased pulse wave velocity associated with reduced calcaneal quantitative osteo-sono index: possible relationship between atherosclerosis and osteopenia. *J Clin Endocrinol Metab*. 2003;88:2573–2578.
16. Salomaa V, Riley W, Kark JD, Nardo C, Folsom AR. Non-insulin-dependent diabetes mellitus and fasting glucose and insulin concentrations are associated with arterial stiffness indexes: the ARIC Study: Atherosclerosis Risk in Communities Study. *Circulation*. 1995;91:1432–1443.
17. Henry RM, Kostense PJ, Spijkerman AM, Dekker JM, Nijpels G, Heine RJ, Kamp O, Westerhof N, Bouter LM, Stehouwer CD. Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study. *Circulation*. 2003;107:2089–2095.
18. Mackey RH, Sutton-Tyrrell K, Vaitkevicius PV, Sakkinen PA, Lyles MF, Spurgeon HA, Lakatta EG, Kuller LH. Correlates of aortic stiffness in elderly individuals: a subgroup of the Cardiovascular Health Study. *Am J Hypertens*. 2002;15:16–23.
19. Levy BI, Ambrosio G, Pries AR, Struijker-Boudier HA. Microcirculation in hypertension: a new target for treatment? *Circulation*. 2001;104:735–740.
20. Baron AD, Brechtel-Hook G, Johnson A, Hardin D. Skeletal muscle blood flow: a possible link between insulin resistance and blood pressure. *Hypertension*. 1993;21:129–135.
21. Bonadonna RC, Saccomani MP, Del Prato S, Bonora E, DeFronzo RA, Cobelli C. Role of tissue-specific blood flow and tissue recruitment in insulin-mediated glucose uptake of human skeletal muscle. *Circulation*. 1998;98:234–241.
22. Serne EH, Stehouwer CD, ter Maaten JC, ter Wee PM, Rauwerda JA, Donker AJ, Gans RO. Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. *Circulation*. 1999;99:896–902.
23. Baumbach GL, Siems JE, Heistad DD. Effects of local reduction in pressure on distensibility and composition of cerebral arterioles. *Circ Res.* 1991;68:338–351.
24. Christensen KL. Reducing pulse pressure in hypertension may normalize small artery structure. *Hypertension*. 1991;18:722–727.
25. James MA, Watt PA, Potter JF, Thurston H, Swales JD. Pulse pressure and resistance artery structure in the elderly. *Hypertension*. 1995;26:301–306.
26. Baumbach GL. Effects of increased pulse pressure on cerebral arterioles. *Hypertension*. 1996;27:159–167.
27. Ryan SM, Waack BJ, Weno BL, Heistad DD. Increases in pulse pressure impair acetylcholine-induced vascular relaxation. *Am J Physiol*. 1995;268:H359–H363.
28. Nigam A, Mitchell GF, Lambert J, Tardif JC. Relation between conduit vessel stiffness (assessed by tonometry) and endothelial function (assessed by flow-mediated dilatation) in patients with and without coronary heart disease. *Am J Cardiol*. 2003;92:395–399.
29. Mitchell GF, Parise H, Vita JA, Larson MG, Warner E, Keaney JF Jr, Keyes MJ, Levy D, Vasan RS, Benjamin EJ. Local shear stress and brachial artery flow-mediated dilation: the Framingham Heart Study. *Hypertension*. 2004;44:134–139.
30. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families: the Framingham offspring study. *Am J Epidemiol*. 1979;110:281–290.
31. Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF Jr, Lehman BT, Fan S, Osypiuk E, Vita JA. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation*. 2004;109:613–619.
32. Mitchell GF, Lacourciere Y, Ouellet JP, Izzo JL Jr, Neutel J, Kerwin LJ, Block AJ, Pfeffer MA. Determinants of elevated pulse pressure in middle-aged and older subjects with uncomplicated systolic hypertension: the role of proximal aortic diameter and the aortic pressure-flow relationship. *Circulation*. 2003;108:1592–1598.
33. Intengan HD, Schiffrin EL. Structure and mechanical properties of resistance arteries in hypertension: role of adhesion molecules and extracellular matrix determinants. *Hypertension*. 2000;36:312–318.
34. Kleinbaum DG, Kupper LL, Muller KE. *Applied Regression Analysis and Other Multivariable Methods*. 2nd ed. Boston, Mass: PWS-Kent Publishing; 1988.
35. *SAS/STAT User's Guide, Version 8*. Cary, NC: SAS Institute; 1999.
36. Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, King GL, LoGerfo FW, Horton ES, Veves A. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes*. 1999;48:1856–62.
37. Shimabukuro M, Higa N, Asahi T, Oshiro Y, Takasu N, Tagawa T, Ueda S, Shimomura I, Funahashi T, Matsuzawa Y. Hypoadiponectinemia is closely linked to endothelial dysfunction in man. *J Clin Endocrinol Metab*. 2003;88:3236–3240.
38. Binggeli C, Spieker LE, Corti R, Sudano I, Stojanovic V, Hayoz D, Luscher TF, Noll G. Statins enhance postischemic hyperemia in the skin circulation of hypercholesterolemic patients: a monitoring test of endothelial dysfunction for clinical practice? *J Am Coll Cardiol*. 2003;42:71–77.
39. Cox RH. Pressure dependence of the mechanical properties of arteries in vivo. *Am J Physiol*. 1975;229:1371–1375.
40. Loutzenhiser R, Bidani A, Chilton L. Renal myogenic response: kinetic attributes and physiological role. *Circ Res*. 2002;90:1316–1324.
41. Baumbach GL, Faraci FM, Heistad DD. Effects of local reduction in pressure on endothelium-dependent responses of cerebral arterioles. *Stroke.* 1994;25:1456–1461.
42. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Kajiyama G, Oshima T. A noninvasive measurement of reactive hyperemia that can be used to assess resistance artery endothelial function in humans. *Am J Cardiol*. 2001;87:121–125.
43. Hayoz D, Weber R, Rutschmann B, Darioli R, Burnier M, Waeber B, Brunner HR. Postischemic blood flow response in hypercholesterolemic patients. *Hypertension*. 1995;26:497–502.
44. Gokce N, Duffy SJ, Hunter LM, Keaney JF, Vita JA. Acute hypertriglyceridemia is associated with peripheral vasodilation and increased basal flow in healthy young adults. *Am J Cardiol*. 2001;88:153–159.
45. Thomas SR, Chen K, Keaney JF Jr. Hydrogen peroxide activates endothelial nitric-oxide synthase through coordinated phosphorylation and dephosphorylation via a phosphoinositide 3-kinase-dependent signaling pathway. *J Biol Chem*. 2002;277:6017–6024.
46. Cryer PE, Haymond MW, Santiago JV, Shah SD. Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. *N Engl J Med*. 1976;295:573–577.
47. Steinsland OS, Furchgott RF. Vasoconstriction of the isolated rabbit ear artery caused by nicotinic agonists acting on adrenergic neurons. *J Pharmacol Exp Ther*. 1975;193:128–137.
48. Grassi G, Seravalle G, Calhoun DA, Bolla GB, Giannattasio C, Marabini M, Del Bo A, Mancia G. Mechanisms responsible for sympathetic activation by cigarette smoking in humans. *Circulation*. 1994;90:248–253.
49. Villablanca AC. Nicotine stimulates DNA synthesis and proliferation in vascular endothelial cells in vitro. *J Appl Physiol*. 1998;84:2089–2098.
50. Heeschen C, Weis M, Cooke JP. Nicotine promotes arteriogenesis. *J Am Coll Cardiol*. 2003;41:489–496.
51. Heeschen C, Weis M, Aicher A, Dimmeler S, Cooke JP. A novel angiogenic pathway mediated by non-neuronal nicotinic acetylcholine receptors. *J Clin Invest*. 2002;110:527–536.
52. Heeschen C, Jang JJ, Weis M, Pathak A, Kaji S, Hu RS, Tsao PS, Johnson FL, Cooke JP. Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. *Nat Med*. 2001;7:833–839.
53. Conklin BS, Zhao W, Zhong DS, Chen C. Nicotine and cotinine up-regulate vascular endothelial growth factor expression in endothelial cells. *Am J Pathol*. 2002;160:413–418.
54. Suner IJ, Espinosa-Heidmann DG, Marin-Castano ME, Hernandez EP, Pereira-Simon S, Cousins SW. Nicotine increases size and severity of experimental choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2004;45:311–317.
55. Zhu BQ, Heeschen C, Sievers RE, Karliner JS, Parmley WW, Glantz SA, Cooke JP. Second hand smoke stimulates tumor angiogenesis and growth. *Cancer Cell*. 2003;4:191–196.
56. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. *J Am Coll Cardiol*. 2004;43:1731–1737.
57. Zatz R, Brenner BM. Pathogenesis of diabetic microangiopathy: the hemodynamic view. *Am J Med*. 1986;80:443–453.
58. Sandeman DD, Shore AC, Tooke JE. Relation of skin capillary pressure in patients with insulin-dependent diabetes mellitus to complications and metabolic control. *N Engl J Med*. 1992;327:760–764.
59. Pinto-Sietsma SJ, Mulder J, Janssen WM, Hillege HL, de Zeeuw D, de Jong PE. Smoking is related to albuminuria and abnormal renal function in nondiabetic persons. *Ann Intern Med*. 2000;133:585–591.