Epidemiology

Cross-Sectional Relations of Multiple Biomarkers From Distinct Biological Pathways to Brachial Artery Endothelial Function

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Background—Endothelial dysfunction is a critical intermediate phenotype in the pathogenesis of cardiovascular disease. We evaluated the relative contributions of distinct biological pathways to interindividual variation in endothelial function by relating prototype biomarkers (representing these pathways) to brachial artery vasodilator function.

Methods and Results—We investigated the cross-sectional relations of a panel of 7 biomarkers measured at a routine examination to brachial artery vasodilator function (flow-mediated dilation [FMD] and reactive hyperemia) assessed at a subsequent examination (mean interval, 2.9 years) in 2113 Framingham Heart Study participants (mean age, 61 years; 54% women). We selected biomarkers from 4 biological domains: neurohormonal (N-terminal pro-atrial natriuretic peptide [N-ANP]), B-type natriuretic peptide [BNP], renin, aldosterone), hemostatic factors (plasminogen activator inhibitor-1 [PAI-1]), inflammation (C-reactive protein [CRP]), and target organ damage (urine albumin-creatinine ratio). In age- and sex-adjusted models, several biomarkers were related to baseline brachial artery diameter (PAI-1, CRP, urine albumin-creatinine ratio), baseline mean flow (N-ANP, BNP, PAI-1, CRP, aldosterone), FMD (N-ANP, PAI-1, CRP, renin), and reactive hyperemia (BNP, PAI-1, CRP, renin, urine albumin-creatinine ratio). In multivariable analyses relating the 7 biomarkers conjointly to each vascular function measure (adjusting for known risk factors), N-ANP and renin were positively related to FMD (P=0.001 and P=0.04, respectively), and N-ANP was inversely related to baseline mean flow velocity (P=0.01). None of the other biomarkers was significantly related to the vascular function measures studied.

Conclusions—In our large community-based sample, a conservative strategy relating several biomarkers to vascular endothelial function identified plasma N-ANP as a key correlate of mean flow under basal conditions and of FMD in response to forearm cuff occlusion. (Circulation. 2006;113:938-945.)

Key Words: atrial natriuretic factor | inflammation | natriuretic peptides | renin

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality in the United States. Experimental and clinical studies indicate that atherosclerotic CVD is a multifactorial disease in which several biological pathways are implicated, including but not limited to the neurohormonal system (natriuretic peptide axis, renin-angiotensin-aldosterone system), hemostatic factors (thrombosis and fibrinolysis), inflammation, and insulin resistance. Classic CVD risk factors may mediate disease hazard by activation of ≥1 of these pathways. Indeed, biomarkers representing each of these pathways, namely N-terminal pro-atrial natriuretic peptide (N-ANP), B-type natriuretic peptide (BNP), renin, aldosterone, plasminogen activator inhibitor-1 (PAI-1), C-reactive protein (CRP), and low-grade albuminuria (urine albumin-creatinine ratio, UACR), have been related prospectively to the risk for clinical CVD events.7-12

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In the past decade, endothelial dysfunction has emerged as an intermediate step along the causal path from standard risk factors to overt CVD. The presence of endothelial dysfunction in turn elevates the risk of CVD. Brachial artery...
flow-mediated dilation (FMD) is a well-studied measure of endothelial function that has been used to noninvasively assess conduit artery and microvascular endothelial function.

We hypothesized, from experimental and physiological data, that several of the aforementioned biological pathways may be related mechanistically to CVD via the induction and promotion of endothelial dysfunction. If so, then prototype biomarkers representing activation of these pathways will be related to measures of endothelial function. Indeed, clinical and epidemiological studies have demonstrated cross-sectional associations of individual biomarkers (representing select biological pathways) with endothelial function and dysfunction. However, the relative contribution of specific pathways to interindividual variation in endothelial function in humans is unclear because no prior investigation has evaluated multiple biomarkers from different biological pathways conjointly.

Accordingly, we investigated the cross-sectional relations of a panel of 7 biomarkers (neurohormones: N-ANP, BNP, renin, aldosterone; hemostatic factor: PAI-1; inflammation: CRP; target organ damage: UACR) to brachial artery vaso-dilator function in a large community-based sample.

Methods

Study Cohort

In 1971, 5124 participants were enrolled in the Framingham Offspring Study as described previously. Participants have been reexamined approximately every 4 years. Participants who attended both the sixth examination (1995 to 1998) and the seventh examination (1998 to 2001) cycles were eligible for the present investigation (n = 3264). We excluded participants for the following reasons: nursing home examination (n = 19), missing covariate data (n = 462), unavailable biomarker (n = 83), or 2D ultrasound data (n = 587). After exclusions, 2113 individuals had data on all vascular function measures. Data on all 7 biomarkers were available for 1764 individuals.

All participants underwent routine medical history, physical examination that included blood pressure measurement, anthropometry, and laboratory assessment of CVD risk factors. Cigarette smoking was defined by self-report of cigarette use within the past 6 hours. Heart rate and blood pressure were measured by a Dinamap automated device (Critikon, Inc). Diabetes was defined as a fasting blood glucose ≥126 mg/dL or use of insulin or oral hypoglycemic agents. CVD was determined by a panel of 3 physicians as previously described. The Institutional Review Board at Boston Medical Center approved the study, and all participants gave written informed consent. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Measurement of Biomarkers

We selected a priori 7 biomarkers for measurement that represented distinct biological domains. Biomarkers were measured from fasting blood samples collected from an antecubital vein during the sixth clinical examination. Participants were in the supine position for 5 to 10 minutes before venipuncture. All samples were obtained in the morning, typically between 8 and 9 AM. Participants were instructed to fast for 12 hours and to take their medications as usual on the morning of the examination. After venous blood was centrifuged at 1850g for 30 minutes, plasma and serum specimens were stored at −70°C until assayed.

Plasma N-ANP and BNP were measured with high-sensitivity immunoradiometric assays (Shionogi). ELISA methods were used to measure plasma levels of PAI-1 antigen according to the method of Declerck et al (TintElize PAI-1, Biopool). High-sensitivity CRP was measured with a Dade Behring BN100 nephelometer. Serum aldosterone was measured with a radioimmunoassay (Quest Diagnostics). Plasma renin was measured by an immunochemilumimetric assay (Nichols assay, Quest Diagnostics). The average interassay coefficients of variation for each biomarker were as follows: N-ANP, 12.7%; BNP, 12.2%; PAI-1, 7.7%; CRP, 2.2%; aldosterone, 4.0% for high concentrations and 9.8% for low concentrations; and renin, 2.0% for high concentrations and 10.0% for low concentrations.

UACR (in mg/g) was measured in spot urine samples (3 mL) collected at the time of the examination and then maintained at −20°C until analysis. Urinary albumin concentration was measured by immunoturbidimetry (Tina-Quant Albumin assay, Roche Diagnostics), and a modified Jaffe method was used to measure urinary creatinine concentration. Average interassay coefficients of variation were 7.2% for urinary albumin and 2.3% for urinary creatinine.

FMD and Reactive Hyperemia Measurements

As described previously, brachial artery FMD (percent change in diameter from baseline: ie, 100 times hyperemia diameter at 1 minute minus baseline diameter divided baseline diameter) and mean hyperemic flow velocity (cm/s) were determined during the seventh clinical examination a mean of 2.9 years after the biomarker determination. Using a Toshiba SSH-140A ultrasound system with a 7.5-MHz linear-array transducer and commercially available software (Brachial Analyzer version 3.2.3, Medical Imaging Applications), investigators who were blinded to participant clinical and biomarker data determined brachial artery diameter at baseline and 1 minute after reactive hyperemia in the brachial artery induced by 5-minute forearm cuff occlusion. Brachial artery diameter was measured as the distance from an intima-lumen boundary to the opposing lumen-intima boundary. The coefficients of variation for baseline and hyperemic diameters were 0.5% and 0.7%, respectively.

Doppler flow was assessed at baseline and during reactive hyperemia with a 3.75-MHz carrier frequency and with correction for the insolation angle. Mean baseline and hyperemic flow velocities were analyzed from digitized audio data with semiautomated signal averaging (Cardiovascular Engineering). Baseline and deflation flow velocity measurements were reproducible on repeated analysis of 30 subjects with correlations >0.98.

Statistical Analyses

All biomarkers displayed skewed distributions and were logarithmically transformed. Age- and sex-adjusted pairwise Pearson correlation coefficients among log-transformed biomarkers (BNP, N-ANP, renin, aldosterone, PAI-1, CRP, and UACR) were calculated.

For this investigation, we focused on 4 measures of vascular function: baseline brachial diameter, FMD, baseline mean flow velocity, and hyperemic mean flow velocity. Age- and sex-adjusted pairwise Pearson correlation coefficients among vascular function measures were estimated. Pearson partial correlation coefficients were used to assess the association of each biomarker with each measure of vascular function, adjusting for age and sex (CORR procedure in SAS).

For the simultaneous consideration of multiple biomarkers in relation to a vascular function measure, we used a conservative 2-step strategy to minimize multiple statistical testing. First, for each vascular variable, we performed a global test of significance to determine whether the group of 7 biomarkers was related to values of that variable. We used linear regression that adjusted for age, sex, and 13 clinical covariates previously reported to be correlated with FMD (ie, mean arterial pressure, brachial artery pulse pressure, heart rate, body mass index, ratio of total to HDL cholesterol, fasting glucose, diabetes, smoking within the past 6 hours, prevalent CVD, hormone replacement therapy, hypertension [systolic blood or diastolic pressure ≥140/90 mm Hg or antihypertensive medication use], lipid-lowering medication, and walk test [before or after FMD determination]). Clinical covariates measured during the seventh examination cycle were used. Second, for vascular function measures that were related to the set of 7 biomarkers with a value of P<0.10, we conducted stepwise multivariable linear regression (SAS REG procedure) with backward selection of biomarkers (adjust-
ing for age, sex, and the 13 clinical covariates that were forced into the model). We considered a 2-sided value of $P<0.05$ as significant.

Because flow velocity is associated with shear stress and artery diameters have been shown to remodel to keep wall shear stress constant (the Glagov phenomenon), we performed secondary analyses in which we included baseline brachial artery diameter as a covariate for 2 measures of vascular function: baseline mean flow velocity and hyperemic mean flow velocity.

We performed additional analyses for those biomarkers identified to be related to vascular function measures in the second step to evaluate effect modification by age (<60 or ≥60 years of age) and obesity (body mass index <30 or ≥30 kg/m²).

## Results

### Participant Characteristics, Biomarkers, and Vascular Function Measures

Study sample characteristics are shown in Table 1 (mean age, 61 years; 54% women). Median (first quartile, third quartile) for the 7 biomarkers and mean (SD) for the 4 vascular function measures are also shown in Table 1. The median time between biomarker and vascular function measurement was 2.9 years.

The 7 biomarkers were only modestly correlated in pairwise comparisons (age-, sex-adjusted partial correlation coefficients ranging from −0.10 to 0.29; Table 2), with the exception of the stronger correlation between the 2 natriuretic peptides (partial correlation coefficient, 0.59). On a parallel note, the 4 measures of vascular function demonstrated a modest to low degree of correlation (age-, sex-adjusted partial correlation coefficients ranging from −0.26 to 0.41; Table 3).

### Correlation of Individual Biomarkers With Measures of Vascular Function

In models adjusted for age and sex, we evaluated whether individual biomarkers were related to 2 measures of conduit artery vasodilator function: baseline diameter and FMD (Table 4). CRP, PAI-1, and UACR were positively related to baseline diameter. We observed a significant positive correlation of N-ANP and renin with FMD. CRP and PAI-1 were inversely related to FMD (Table 4).

We examined the relations between individual biomarkers and 2 measures of forearm microvascular vasodilator function: baseline mean flow velocity and hyperemic mean flow velocity (Table 4). We observed a significant inverse relation between N-ANP and BNP with baseline mean flow. CRP, PAI-1, and aldosterone were all positively related to baseline flow. Several biomarkers were significantly related to hyper-

### TABLE 1. Characteristics of the Study Sample

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men (n=975)</th>
<th>Women (n=1138)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>61±10</td>
<td>61±9</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>126±17</td>
<td>121±18</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>75±10</td>
<td>66±11</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.7±4.6</td>
<td>27.4±5.7</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>50</td>
<td>42</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Biomarkers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-ANP, pmol/L</td>
<td>281.0 (196.0, 428.0)</td>
<td>343.0 (248.0, 482.0)</td>
</tr>
<tr>
<td>BNP, pg/mL</td>
<td>6.0 (4.0, 15.2)</td>
<td>9.4 (4.0, 18.7)</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.8 (0.9, 3.6)</td>
<td>2.3 (1.0, 5.5)</td>
</tr>
<tr>
<td>PAI-1, ng/mL</td>
<td>26.1 (17.3, 36.5)</td>
<td>19.8 (11.7, 31.2)</td>
</tr>
<tr>
<td>Aldosterone, ng/dL</td>
<td>9.0 (7.0, 13.0)</td>
<td>10.0 (7.0, 15.0)</td>
</tr>
<tr>
<td>Renin, μU/mL</td>
<td>14.0 (8.0, 25.0)</td>
<td>11.0 (6.0, 19.0)</td>
</tr>
<tr>
<td>UACR, mg/g</td>
<td>4.8 (2.2, 11.0)</td>
<td>8.5 (3.6, 16.7)</td>
</tr>
<tr>
<td>Measures of vascular function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline brachial diameter, mm</td>
<td>4.94 (0.65)</td>
<td>3.68 (0.57)</td>
</tr>
<tr>
<td>FMD, %</td>
<td>2.28 (2.41)</td>
<td>3.24 (3.01)</td>
</tr>
<tr>
<td>Baseline mean flow velocity, cm/s</td>
<td>8.58 (5.13)</td>
<td>7.73 (4.77)</td>
</tr>
<tr>
<td>Hyperemic mean flow velocity, cm/s</td>
<td>47.1 (20.0)</td>
<td>53.5 (21.6)</td>
</tr>
</tbody>
</table>

For continuous measures, values are mean±SD; categorical variables, percentages; and biomarkers, median (first, third quartiles).

### TABLE 2. Age- and Sex-Adjusted Correlations Among Biomarker Levels

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>BNP</th>
<th>CRP</th>
<th>PAI-1</th>
<th>Aldosterone</th>
<th>Renin</th>
<th>UACR</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-ANP</td>
<td>0.58</td>
<td>−0.05</td>
<td>−0.19</td>
<td>−0.19</td>
<td>−0.18</td>
<td>0.01</td>
</tr>
<tr>
<td>BNP</td>
<td>...</td>
<td>−0.04</td>
<td>−0.10</td>
<td>−0.16</td>
<td>−0.16</td>
<td>0.05</td>
</tr>
<tr>
<td>CRP</td>
<td>...</td>
<td>...</td>
<td>0.29</td>
<td>0.11</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>PAI-1</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.10</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.21</td>
<td>0.09</td>
</tr>
<tr>
<td>Renin</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.08</td>
</tr>
</tbody>
</table>

All biomarkers were log transformed. For all correlations, $P<0.05$, except (BNP, CRP), (N-ANP, UACR), and (PAI-1, UACR). n=1764.
Conjoint Relations of Multiple Biomarkers and Measures of Vascular Function

The global test evaluating whether the group of 7 biomarkers was related to measures of vascular function was significant for the FMD phenotype \((P=0.02; \text{Table 5})\). To identify the multivariable correlates of FMD, we conducted backward selection, including the 7 biomarkers after adjusting for age, sex, and 13 clinical covariates. In the final model, N-ANP and renin remained significantly and positively associated with FMD \((P=0.001\) and \(P=0.04\), respectively; \text{Table 5})

Secondary Analyses

By incorporating an interaction term in a multivariable model, we evaluated whether the relation between FMD and N-ANP or renin was modified by age or obesity and did not find effect modification by either age or obesity for either biomarker. Because we did not observe significant correlations of the panel of biomarkers with baseline diameter and hyperemic flow, we assessed our statistical power to detect associations with a biomarker. We had \(80\%\) power at an \(\alpha=0.05\) to detect very small effect sizes: a contribution of 0.29\% or 0.57\% to interindividual variation in baseline diameter or FMD, respectively, and 0.59\% or 0.48\% for baseline or hyperemic flow velocity, respectively.\(^{30}\)

Discussion

Principal Findings

In a community-based sample, we jointly examined the relations of a panel of 7 biomarkers representing distinct biological axes to conduit brachial artery function assessed via FMD and to Doppler flow velocities that are influenced by microvascular function and overall arterial impedance. We observed that N-ANP and renin were positively related to FMD and that N-ANP was inversely related to baseline mean flow velocity. In multivariable analyses relating the 7 biomarkers conjointly to each vascular function measure (adjusting for known risk factors), none of the other biomarkers was significantly related to any vascular function measure.

Natriuretic Peptides and Endothelial Function

In addition to diuresis and natriuresis, a relaxant effect on vascular smooth muscle was among the earliest properties demonstrated for natriuretic peptides by in vitro studies.\(^{31}\) The vasodilator properties of natriuretic peptides have been attributed to 2 mechanisms: an effect of binding to natriuretic peptide receptor A and a direct endothelium-dependent stimulation via nitric oxide.\(^{32}\) Natriuretic peptide receptors are linked to the particulate guanylyl cyclase, and like nitric oxide, N-ANP and BNP produce vasodilation by the cGMP-dependent signaling cascade. Some prior studies of healthy volunteers have assessed the in vivo effect of natriuretic peptides on endothelial function by infusing N-ANP or BNP and examining brachial artery FMD and forearm blood flow.\(^{16,33}\) In these studies, both N-ANP and BNP have been shown to lead to vasodilation, with equimolar doses of N-ANP inducing significantly greater vasodilation than BNP. Our results demonstrating a positive relation of N-ANP to FMD extend these observations to a community-based sample.

It is important to note that the findings of the present study differ from results of prior studies reporting an inverse relation between natriuretic peptides and FMD in patients with congestive heart failure.\(^{34}\) That finding might be expected because congestive heart failure is associated with elevated natriuretic peptide levels and impaired FMD. The apparent discrepancy between those findings and the present study is likely attributable to differences in the study samples. Prior studies have examined a diseased sample, whereas the present study examined a relatively healthier sample with a low prevalence of CVD.

### TABLE 3. Age- and Sex-Adjusted Correlations Among Vascular Function Measures

<table>
<thead>
<tr>
<th>Measures of Vascular Function</th>
<th>FMD</th>
<th>Baseline Mean Flow Velocity</th>
<th>Hyperemic Mean Flow Velocity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline brachial diameter</td>
<td>-0.26</td>
<td>0.009</td>
<td>-0.17</td>
</tr>
<tr>
<td>FMD</td>
<td>...</td>
<td>0.08</td>
<td>0.41</td>
</tr>
<tr>
<td>Baseline mean flow velocity</td>
<td>...</td>
<td>...</td>
<td>0.27</td>
</tr>
</tbody>
</table>

\(^*\)For all correlations, \(P<0.05\), except (baseline brachial diameter, baseline mean flow). \(n=2113\).

### TABLE 4. Relations Between Biomarkers From Distinct Pathways and Measures of Vascular Function

<table>
<thead>
<tr>
<th>Age- and Sex-Adjusted Pearson Partial Correlation Coefficients*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-ANP</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Baseline diameter</td>
</tr>
<tr>
<td>FMD</td>
</tr>
<tr>
<td>Baseline mean flow</td>
</tr>
<tr>
<td>Hyperemic mean flow</td>
</tr>
</tbody>
</table>

\(*\)Data are partial correlations \(r\).

All biomarkers were natural log-transformed.
It is noteworthy that N-ANP was inversely related to baseline mean flow velocity. A recent animal study demonstrated that deletion of the receptor for ANP in the mouse causes a marked increase in plasma volume and cardiac output.35 We speculate that higher baseline flow in the forearm in patients with low ANP levels might reflect a loss of the natriuretic and hypovolemic effects of ANP.

**TABLE 5. Joint Consideration of Multiple Biomarkers in Relation to Each Vascular Function Measure**

<table>
<thead>
<tr>
<th>Biomarker†</th>
<th>β‡ (SE)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-ANP</td>
<td>0.442 (0.137)</td>
<td>0.001</td>
</tr>
<tr>
<td>Renin</td>
<td>0.140 (0.067)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*For vascular function measures with a global P<0.10, individual biomarkers significantly related to vascular function measures after stepwise selection are displayed.

<table>
<thead>
<tr>
<th>Global P</th>
<th>Stepwise Selection Biomarker†</th>
<th>β‡ (SE)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline diameter</td>
<td>0.65</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>FMD</td>
<td>0.02</td>
<td>N-ANP</td>
<td>0.442 (0.137)</td>
</tr>
<tr>
<td>Baseline mean flow velocity</td>
<td>0.08</td>
<td>N-ANP</td>
<td>−0.617 (0.244)</td>
</tr>
<tr>
<td>Hyperemic mean flow velocity</td>
<td>0.49</td>
<td>…</td>
<td>…</td>
</tr>
</tbody>
</table>

A test of whether any of 7 biomarkers (N-ANP, BNP, CRP, PAI-1, aldosterone, renin, UACR) differed with respect to vascular function dependent measures. Covariates in the multivariable models included age, sex, mean arterial pressure, brachial artery pulse pressure, heart rate, body mass index, ratio of total to HDL cholesterol, fasting glucose, diabetes, smoking within the past 6 hours, prevalent cardiovascular disease, hormone replacement therapy, hypertension medication, lipid-lowering medication, and walk test.

†β, the regression coefficient, indicates a change in vascular function measure per 1-SD increment in log-marker. 1 SD of log N-ANP is 0.56 (an SD increment indicates a 1.75-fold increase in levels in original units); 1 SD of log renin is 0.98 (an SD increment indicates a 2.67-fold increase in levels in original units).

**PAI-1 and Endothelial Function**

PAI-1 is largely produced by endothelial cells, and increased PAI-1 production and a consequent increase in thrombogenicity are accepted as important components of endothelial dysfunction.36 Few prior studies, however, have related plasma PAI-1 levels to measures of vascular function.17,37 One such study of 35-year-old men and women (n=109)
observed an inverse correlation between plasma PAI-1 levels and FMD in unadjusted analyses. This relationship was not significant after adjustment for clinical covariates. In age- and sex-adjusted analyses, we likewise observed an inverse correlation between plasma PAI-1 and both FMD and hyperemic flow. These correlations were rendered nonsignificant on adjustment for traditional cardiovascular risk factors. Our results are consistent with the notion that plasma PAI-1 may be a marker of endothelial dysfunction but the relations are confounded by other risk factors.

Aldosterone, Renin, and Endothelial Function
In vitro studies have demonstrated conflicting results with regard to the effects of aldosterone on endothelial function. Aldosterone has been reported both to decrease\(^{39}\) and to increase\(^{39}\) the expression of inducible nitric oxide synthase in experimental studies. In patients with hypertension, congestive heart failure,\(^{40}\) and hyperaldosteronism,\(^{18}\) circulating aldosterone levels have been associated with impaired FMD, and such impairment has been shown to improve after treatment with an aldosterone antagonist. We did not observe any association of serum aldosterone and vascular function in our relatively healthy sample.

The positive association of plasma renin with FMD is intriguing. Consistent with our findings, Duffy and colleagues\(^{41}\) recently found that low renin hypertension was associated with a marked impairment in nitric oxide–mediated vasodilation of resistance vessels. However, our current observations conflict with some prior reports suggesting that some conditions associated with elevated renin levels (such as renovascular hypertension)\(^ {42}\) are associated with endothelial dysfunction. It is conceivable that differences in samples (our sample was relatively healthy) may account for these differences. Additionally, it is noteworthy that the relations of renin to nitric oxide synthase are controversial in the published literature. Nitric oxide has been reported both to stimulate\(^ {43}\) and to suppress\(^ {44}\) renin levels. Additional studies are warranted to corroborate/refute our findings.

CRP and Endothelial Function
In a prior study involving the Framingham offspring sample, we observed that inflammation, as assessed by multiple inflammatory markers, including CRP measured at the same examination as vascular function (examination 7), was inversely related to measures of endothelial function.\(^ {21}\) Those relations were markedly weakened after the adjustment for traditional cardiovascular risk factors. The results of the present study are consistent with this previous observation. The results of both studies combined suggest that risk factors induce a state of systemic inflammation that impairs endothelial function, particularly in the brachial artery.

Urine Albumin Excretion and Endothelial Function
In small numbers of patients with diabetes or hypertension, microalbuminuria has been associated with endothelial dysfunction as assessed by the plasma biomarker von Willebrand factor.\(^ {19,45}\) Meanwhile, in apparently healthy individuals, microalbuminuria has been inconsistently associated with FMD.\(^ {46,47}\) We did not observe a significant association between microalbuminuria and FMD. It has been suggested that UACR is indicative of microvascular endothelial dysfunction and may not be related to indicators of macrovascular endothelial function (FMD).\(^ {47}\)

Joint Consideration of Multiple Biomarkers and Endothelial Function
Using a multiple biomarker approach, we sought to clarify the relative contribution of specific biological pathways to inter-individual variation in endothelial function. Among 7 biomarkers evaluated, we identified N-ANP as the strongest correlate of brachial artery endothelial function. This observation raises several possibilities. First, a possible interpretation of our findings is that the natriuretic peptide axis is the strongest contributor to endothelial function in the community. Both animal and in vitro studies support this interpretation; they have shown that natriuretic peptides potently vasodilate blood vessels. Second, we observed that several other biomarkers were related to endothelial function in age- and sex-adjusted models (but not in multivariable models), suggesting that these pathways may be important correlates of endothelial function but that the association may be mediated via known CVD risk factors. Finally, these other biomarkers and the pathways they represent may affect atherosclerosis by mechanisms other than endothelial dysfunction.

Study Strengths and Limitations
The large sample size, routine assessment of 7 biomarkers, routine measurement of 4 measures of vascular function, availability of standardized clinical covariates, use of multivariable analyses, and use of a community-based sample strengthen the present investigation. Nonetheless, it is important to acknowledge several limitations of our approach. First, the biomarkers were assessed an average of 2.9 years before the vascular measures; hence, we cannot exclude the possibility that contemporaneous biomarkers may have been more closely associated with vascular function measures. Second, the lack of biomarker and vascular function assessments at 2 time points precludes any causal inferences with regard to the association between the measures. Third, for certain biomarkers, measurement-related issues may have limited our power to detect a relation. For plasma BNP levels, a significant proportion of individuals (25% of women, 38% of men)\(^7\) were below the detection limit of the assay. For plasma renin and serum aldosterone, we considered a single-occasion measurement obtained on a random sodium diet and without the period of supine rest typically advocated for these biomarkers in clinical settings; it may be questioned whether a single reading thus obtained adequately represents an individual’s renin and mineralocorticoid profile. Fourth, the mean FMD was lower in our sample compared with some prior studies, probably because of the age of the sample and the below-the-elbow cuff position.\(^ {48}\) However, the FMD results are consistent with other studies with a comparable cuff position.\(^ {49}\) Fifth, although the associations between N-ANP and renin and vascular measures were statistically robust, the magnitude of the associations was quite modest. As such, these data provide primarily mechanistic insights about the relative contribution of biological pathways to
endothelial function; the clinical relevance of these observations remains to be established. Finally, given the predominantly white and middle-aged to elderly composition of our sample, the generalizability of our findings to other ethnicities and younger individuals is unknown.

Implications and Conclusions

The availability of an increasingly vast number of biomarkers has resulted in a plethora of clinical studies describing the relations of ≥1 biomarkers to specific intermediate and clinical phenotypes. Reports evaluating individual biomarkers cannot provide insights into the relative contributions of each marker when considered in the context of others. A related challenge is one of multiple statistical testing. If one tests several biomarkers in relation to ≥1 phenotypes, some biomarkers may be related to select phenotypes by chance alone.

The present investigation provides a conservative scientific and analytic approach by examining the relations of a panel of 7 biomarkers to 4 vascular function phenotypes using a “global” statistical test. Additionally, we have presented the results of standard individual biomarker analyses so that our data can be compared with prior research. We selected biomarkers to represent biologically relevant axes based on experimental and clinical data. However, we acknowledge that the panel of biomarkers will always be somewhat arbitrary, based on practical constraints (it may not be feasible for any given study to examine all biomarkers), and that the biomarkers for inclusion will vary over time with advancements in our knowledge. Nonetheless, we believe that this analytic framework may be adapted to multimarker investigations of CVD phenotypes.

In summary, a conservative assessment of a panel of biomarkers in relation to measures of vascular function in our large community-based sample demonstrated that plasma N-ANP is a key correlate of mean flow under basal conditions and of FMD in response to forearm cuff occlusion. Additional studies are warranted to confirm our findings.

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Disclosures

Dr. Mitchell owns stock in 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 drugs and interventions on vascular stiffness. No other author has any ownership rights or other financial relationship with Cardiovascular Engineering. 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


**Clinical Perspective**

Several biological pathways have been implicated in the pathogenesis of atherosclerotic cardiovascular disease (CVD), and biomarkers representing activation of these pathways are measurable in blood. Endothelial dysfunction is a key intermediate step along the causal path from atherosclerotic risk factors to clinical CVD. We hypothesized that circulating biomarkers of several atherosclerosis-related pathways will be related to endothelial function. In a community-based sample, we tested our hypothesis by relating a panel of 7 biomarkers (representing neurohormonal, inflammatory, hemostatic pathways, and target organ damage) to noninvasive measures of brachial artery endothelial function. We observed that plasma N-terminal pro-atrial natriuretic peptide and renin were positively related to brachial artery flow-mediated dilation and that N-terminal pro-atrial natriuretic peptide was inversely related to baseline mean flow velocity. Our cross-sectional results are consistent with the notion that the natriuretic peptide system influences endothelial function in the community. Further research is needed to confirm our findings. Additionally, our report illustrates an analytic approach for evaluating the relations of multiple biomarkers to CVD phenotypes.
Cross-Sectional Relations of Multiple Biomarkers From Distinct Biological Pathways to Brachial Artery Endothelial Function

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In the first online posting of the article “Cross-Sectional Relations of Multiple Biomarkers From Distinct Biological Pathways to Brachial Artery Endothelial Function” by Kathiressan et al (http://circ.ahajournals.org/cgi/content/short/CIRCULATIONAHA.105.580233v1), there was an incorrect value in Table 4. The relation between hyperemic mean flow and CRP was incorrectly listed as $-1.07$. The correct value, which is $-0.07$, appears in print (Circulation. 2006;113:938–945) and in the current online version (http://circ.ahajournals.org/cgi/content/full/113/7/938).

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