Vascular Endothelial Dysfunction and Mortality Risk in Patients With Chronic Heart Failure

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Background—Endothelial function is known to be impaired in subjects with chronic heart failure (CHF), but the association between endothelial function and subsequent mortality risk in CHF has not been previously reported.

Methods and Results—Biomarkers of endothelial function in the systemic arterial circulation (flow-mediated dilation [FMD] in the brachial artery) and the pulmonary circulation (exhaled nitric oxide [NO] production during submaximal exercise) were prospectively assessed in 259 subjects with New York Heart Association class II–III CHF. In subjects with FMD measurements (n=149), there were 12 deaths and 5 urgent transplantations over a median follow-up period of 841 days. In subjects with exhaled NO production measurements (n=110), there were 18 deaths and 1 urgent transplantation over a median follow-up period of 396 days. Both decreased FMD and decreased exhaled NO production were associated with increased risk of death or urgent transplantation after adjustment for other known CHF prognostic factors (age, etiology of CHF, functional class, left ventricular ejection fraction) in Cox multivariate proportional-hazards models (adjusted hazard ratio [HR] estimate for a 1% decrease in FMD 1.20; 95% confidence interval [CI], 1.03 to 1.45; P=0.027; adjusted HR estimate for a 1-ppb/min decrease in exhaled NO production 1.31, 95% CI, 1.01 to 1.69, P=0.04).

Conclusions—Endothelial dysfunction in CHF, as assessed by FMD in the brachial artery and exhaled NO production during submaximal exercise, is associated with an increased mortality risk in subjects with both ischemic and nonischemic CHF. (Circulation. 2005;111:310-314.)

Key Words: nitric oxide ■ endothelium ■ heart failure ■ survival

Endothelium-dependent, nitric oxide (NO)–mediated vasodilation in response to hormonal agonists and/or shear stress is decreased in the skeletal muscle, coronary, and pulmonary circulations of patients with chronic heart failure (CHF) when compared with healthy subjects.1–3 Impaired endothelium-dependent vasodilation in patients with CHF is attributable to decreased activity of the L-arginine–NO synthetic pathway, increased degradation of NO by reactive oxygen species, and hyporesponsiveness in vascular smooth muscle.4–6 Impaired endothelial function is known to be associated with reduced exercise hyperemia and impaired functional capacity,7,8 but its association with clinical outcomes has not been previously reported.

The present study was undertaken to prospectively determine the association of 2 biomarkers of endothelial function, flow-mediated dilation (FMD) in the brachial artery and exhaled NO production during submaximal exercise, and subsequent mortality risk in subjects with CHF.

Study Population
Two hundred fifty-nine ambulatory subjects with CHF were sequentially recruited in 2 separate cohorts: 149 subjects for determination of FMD measurements and 110 subjects for determination of exhaled NO production during submaximal exercise. Eligible subjects had stable New York Heart Association (NYHA) functional classification II–III symptoms ≥3 months and left ventricular ejection fraction ≤40%. Patients with exercise-limiting coronary artery disease or peripheral vascular disease, renal disease (serum creatinine >3.0 mg/dL), liver disease (liver enzyme tests >3 times the upper limit of normal), chronic lung disease, or chronic inflammatory diseases were excluded. Background therapy with digoxin, diuretics, angiotensin-converting enzyme inhibitors, nitrates, and/or β-adrenergic receptor blockers was discontinued on the morning of testing. Study protocols were approved by the Columbia University and Yale University Ethical Review Committees. All subjects provided written, informed consent before participation.

Brachial Artery Ultrasound Imaging
Endothelium-dependent, flow-mediated dilation of the brachial artery was determined with high-resolution ultrasound imaging (Ad...
vanced Technology Laboratories) adapted from published guidelines as previously described. 

Brachial artery diameter was determined as the mean of 5 measurements from a single cardiac cycle at end-diastole at rest and 60 to 75 seconds after release of a 5-minute forearm cuff occlusion. FMD was calculated as the percent change in brachial artery diameter after forearm cuff release compared with the resting brachial artery diameter. Brachial artery blood flow velocity (cm/s) was determined with a 1.2-mm, pulsed-Doppler sampling volume in the vessel lumen midline with software correction for the incident angle of 60° at rest and for the first 15 seconds after forearm cuff release. The mean blood flow velocity over 5 cardiac cycles is reported.

**Determination of NO Production in Expired Gases**
Subjects breathed by mouth through a low-resistance, 3-way valve from a 500-L reservoir of NO-free air with a nose clip to exclude nasopharyngeal sources of NO. In a preliminary study of 10 CHF subjects, inhalation of aerosolized N\textsuperscript{3}-monomethyl-L-arginine did not alter exhaled NO production when compared with placebo, confirming a lower-airway source for exhaled NO in these patients. NO in expired gases (ppb) was measured with a calibrated chemiluminescent NO analyzer (Sievers model 280). Ventilation (L/min) was simultaneously measured with a calibrated pneumotachometer. NO production (ppb/min) was calculated as the product of the phase-corrected NO concentration and the ventilation signals. Because minute ventilation has been previously reported to influence NO mass transfer from alveoli, all exhaled NO production measurements were determined at submaximal work rates individually adjusted to correspond to a standardized minute ventilation of 20 L/min. Subjects continued steady-state submaximal exercise at the target work rate for 5 minutes until a plateau in NO production was present. The average exhaled NO production in the last minute of exercise is reported.

**Patient Outcomes Determination**
Patient outcomes (death, urgent cardiac transplantation, or no event) were determined for all patients by direct contact with the patient, his/her family, or physician. Subjects of the cohort with exhaled NO production measurements were enrolled between January 1998 and January 1999, and vital status was determined for all subjects of the cohort as of August 1, 1999. Subjects of the cohort with FMD measurements were enrolled between May 2000 and January 2003, and vital status was determined for all subjects of the cohort as of May 16, 2003.

**Data Analysis**
All values are presented as mean±SEM. Survival data were analyzed by the Kaplan-Meier method, log-rank tests for univariate analyses, and Cox proportional-hazards models for multivariate analyses (Stata biostatistical software, version 8.0). Known prognostic indicators (age, etiology of CHF, NYHA class, left ventricular ejection fraction) were included in all Cox multivariate models. Proportional-hazards assumptions were tested in final models based on analysis of Schoenfeld residuals. With an assumed annual event rate of 20%, 1-year accrual with 2-year follow-up, and 2-tailed α=0.05, the power to detect 2-fold and 3-fold risk differences in groups above and below the median value was 63% and 94%, respectively, for the 110 subjects with exhaled NO production measurements and 76% and 98%, respectively, for the 149 subjects with FMD measurements. For all analyses, a probability value <0.05 was used to infer statistical significance.

**Results**
Clinical characteristics of survivors and nonsurvivors are provided in Table 1. There were 12 deaths and 5 urgent transplantations over a median follow-up period of 841 days. When grouped by FMD responses above or below the median value of 1.79%, subjects with lower values of FMD had significantly more events (n=13) than did subjects with higher values of FMD (n=4, Figure 1; P=0.01, log-rank test). In Cox proportional-hazards models adjusted for other
Exhaled NO, ppb/min 4.3
endothelial function, as evidenced by decreased FMD in the
The present findings demonstrate that impaired vascular
values of NO production had more events (n
below the median value of 4.1 ppb/min, subjects with lower
mortality risk when considered as a continuous variable (HR estimate for a 1-ppb/min decrease in FMD
significantly associated with increased mortality risk when considered as a continuous
Clinical characteristics of survivors and nonsurvivors are
TABLE 2. Clinical Characteristics of 110 Subjects With
Measurements of Exhaled NO Production During
Submaximal Exercise
<table>
<thead>
<tr>
<th>Age, y</th>
<th>All Subjects (n=110)</th>
<th>Survivors (n=91)</th>
<th>Nonsurvivors (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>58±1</td>
<td>58±1</td>
<td>58±3</td>
<td></td>
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<tr>
<td>Sex, % male</td>
<td>82</td>
<td>80</td>
<td>89</td>
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<tr>
<td>Etiology, % ischemic</td>
<td>56</td>
<td>53</td>
<td>57</td>
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<tr>
<td>NYHA class, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class II</td>
<td>54</td>
<td>54</td>
<td>53</td>
</tr>
<tr>
<td>Class III</td>
<td>46</td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>0.25±0.01</td>
<td>0.25±0.01</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>Comorbidities, %</td>
<td></td>
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<tr>
<td>Diabetes</td>
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<td>33</td>
<td>37</td>
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<tr>
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<tr>
<td>Never</td>
<td>40</td>
<td>41</td>
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</tr>
<tr>
<td>Past history</td>
<td>56</td>
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<td>61</td>
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<tr>
<td>Current</td>
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<td>4</td>
<td>7</td>
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<td>Medications, %</td>
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<td>RAS inhibitors</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>β-Blockers</td>
<td>56</td>
<td>55</td>
<td>63</td>
</tr>
<tr>
<td>Nitrates</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Exhaled NO, ppb/min</td>
<td>4.3±0.19</td>
<td>4.5±0.22</td>
<td>3.6±0.43</td>
</tr>
<tr>
<td>Ventilation, L/min</td>
<td>21±0.6</td>
<td>21±0.7</td>
<td>22±1.2</td>
</tr>
</tbody>
</table>

Exhaled NO Production Cohort
Clinical characteristics of survivors and nonsurvivors are provided in Table 2. There were 18 deaths and 1 urgent transplantation over a median follow-up period of 396 days. When grouped by exhaled NO production responses above or below the median value of 4.1 ppb/min, subjects with lower values of NO production had more events (n=13) than did subjects with higher values of NO production (n=6; P=0.05, by log-rank test). In Cox proportional-hazards models adjusted for other known prognostic factors, decreased exhaled NO production was significantly associated with increased mortality risk when considered as a continuous variable (HR estimate for a 1-ppb/min decrease in exhaled NO production=1.31; 95% CI, 1.01 to 1.69; P=0.04) or dichotomized at the median value (HR estimate for below median exhaled NO production=2.82; 95% CI, 1.04 to 7.63; P=0.04).

Discussion
The present findings demonstrate that impaired vascular endothelial function, as evidenced by decreased FMD in the
brachial artery and decreased exhaled NO production during submaximal exercise, is associated with increased mortality risk in CHF subjects after adjustment for other known clinical prognostic factors.

Previous experimental and clinical studies have demonstrated impaired endothelium-dependent vasodilation in coronary, skeletal muscle, and pulmonary circulations in patients with CHF.1–3,15,16 In the present study, FMD was used as an indirect biomarker of endothelial function, because this response is partly dependent on shear stress-induced release of NO.17 FMD is also regulated by local prostaglandin production, by the effects of local metabolic conditions on hemoglobin-NO interactions, and by systemic effects related to inflammation, sympathetic activation, and deconditioning.18–23 The present study design cannot determine which regulatory component(s) of the FMD response might be linked to disease progression in CHF. The relative roles of endothelial cell dysfunction and vascular smooth muscle dysfunction cannot be discerned from our study, because nitroglycerin responses were not recorded. FMD in the brachial artery is closely associated with endothelium-dependent vasomotion in the coronary circulation and is thought to be associated with proinflammatory and prothrombotic changes in endothelial cell phenotype.24–26 In subjects with ischemic CHF, the association between endothelial dysfunction and mortality could be related to an increased risk of ischemic events, as has been previously described in patients with coronary artery disease and peripheral vascular disease.27 Other mechanisms are also likely contributory, because the observed association between endothelial function and mortality was independent of CHF etiology. Endothelial dysfunction in the coronary microcirculation could contribute to disease progression by inducing local areas of ischemia and/or necrosis.28 Endothelial dysfunction may be also be linked to vasculature-dependent changes in ventricular loading conditions that could promote ventricular remodeling and disease progression.29

Figure 2. Kaplan-Meier plot showing proportion of survivors over time in subjects with exhaled NO production above (dashed line) and below (solid line) median value of 4.1 ppb/min. Subjects with lower values of NO production had more events (n=13) than did subjects with higher values of NO production (n=6; P=0.05, by log-rank test). Abbreviations are as defined in text.
NO increases in response to exercise in the healthy human and is thought to play an important role in the normal regulation of smooth muscle tone in pulmonary airways and blood vessels.\textsuperscript{11,30–33} Experimental and clinical studies have demonstrated that NO may be derived from endothelial, epithelial, and inflammatory cells present throughout the respiratory tract.\textsuperscript{11,34–36} Studies in isolated lung preparations and the intact human respiratory tract have demonstrated that a portion of NO in expired gases is derived from the lower respiratory tract, including alveolae.\textsuperscript{37–40} Increased exhaled NO production in response to exercise may be attributed to increased shear stress–induced release of NO from alveolar endothelial cells and increased exercise hyperventilation-dependent mass transport of NO from alveoli.\textsuperscript{11,41,42} Airway inflammation may also contribute to exhaled NO production.\textsuperscript{36,43,44} In patients with CHF, decreased exhaled NO production during exercise is associated with increased pulmonary vascular resistance and decreased maximal aerobic capacity.\textsuperscript{41,45–48} Decreased exhaled NO production in patients with CHF may be attributed to decreased diffusion/transport of NO from lower-airway sources and/or a reduced rate of cellular production of NO in the lower respiratory tract.\textsuperscript{4,40,49–52} Increased mortality risk in association with decreased exhaled NO production could be related to autonomic dysregulation of ventilation during exercise, progression of right ventricular dysfunction in response to increased pulmonary vascular resistance, or other hemodynamic factors associated with decreased aerobic capacity.\textsuperscript{41,47,48,53}

Interpretation of our findings is potentially limited by several considerations. Although our findings with 2 biomarkers of endothelial function and subsequent mortality risk are internally consistent in our study cohorts, the observational nature of the study does not allow one to draw conclusions regarding a causal link between endothelial dysfunction and mortality, because unmeasured confounders may have contributed to the observed associations. Our study sample was recruited from a tertiary referral heart failure and transplantation center and therefore may not be representative of CHF patients in the general community. The sample size and relatively small number of events limited the number of variables in our regression models and provided sufficient statistical power to detect only large risk differences with wide CIs. Although our reported associations were adjusted for several known prognostic factors, we were unable to adjust our findings for other important prognostic indicators, including left ventricular end-diastolic diameter, peak aerobic capacity, implantable cardioverter-defibrillator use, and biomarkers of neurohormonal activation, because these data were unavailable for all of our subjects. Additional work in a larger study sample is warranted to determine whether endothelial dysfunction is independently associated with mortality after adjustment for these other known prognostic factors. Additional data on the association between endothelial function and cardiovascular disease–specific mortality and major cardiac disease–related morbidities, including nonfatal myocardial infarction and hospitalization for CHF, are also needed. Although discontinued for 12 to 24 hours before all study measurements, background medications may have contributed to our findings.

In conclusion, these data provide the first evidence of an association between markers of endothelial function and subsequent clinical outcomes in patients with CHF.Additional work is needed to determine the underlying mechanisms and potential therapeutic implications of these findings.

Acknowledgments

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