Vascular Dysfunction and Chronic Obstructive Pulmonary Disease
The Role of Redox Balance


Abstract—Chronic obstructive pulmonary disease (COPD) is characterized by low pulmonary function, inflammation, free radical production, vascular dysfunction, and subsequently a greater incidence of cardiovascular disease. By administering an acute oral antioxidant cocktail to patients with COPD (n=30) and controls (n=30), we sought to determine the role of redox balance in the vascular dysfunction of these patients. Using a double-blind, randomized, placebo-controlled, crossover design, patients with COPD and controls were ingested placebo or the antioxidant cocktail (vitamin C, vitamin E, α-lipoic acid) after which brachial artery flow-mediated dilation and carotid-radial pulse wave velocity were assessed using ultrasound Doppler. The patients exhibited lower baseline antioxidant levels (vitamin C and superoxide dismutase activity) and higher levels of oxidative stress (thiobarbituric acid reactive species) in comparison with controls. The patients also displayed lower basal flow-mediated dilation (P<0.05), which was significantly improved with antioxidant cocktail (3.1±0.5 versus 4.7±0.6%; P<0.05; placebo versus antioxidant cocktail), but not controls (6.7±0.6 versus 6.9±0.7%; P>0.05; placebo versus antioxidant cocktail). The antioxidant cocktail also improved pulse wave velocity in patients with COPD (14±1 versus 11±1 m·s$^{-1}$; P<0.05; placebo versus antioxidant cocktail) while not affecting controls (11±2 versus 10±1 m·s$^{-1}$; P>0.05; placebo versus antioxidant). Patients with COPD exhibit vascular dysfunction, likely mediated by an altered redox balance, which can be acutely mitigated by an oral antioxidant. Therefore, free radicals mediated vascular dysfunction may be an important mechanism contributing to this population’s greater risk and incidence of cardiovascular disease. (Hypertension. 2014;63:00-00.)

Key Words: free radicals, oxidative stress, pulmonary disease, chronic obstructive, vascular dysfunction

Chronic obstructive pulmonary disease (COPD) is a condition that originates in the pulmonary system but is now well recognized to manifest as a syndrome encompassing other symptomatology and comorbidities beyond pulmonary disease, most of which seem to be vascular related. Specifically, an increased incidence of hypertension, advanced atherosclerosis, coronary artery disease, peripheral vascular disease, and elevated cardiovascular mortality are now often considered hallmarks of COPD. However, the mechanistic link among COPD, vascular dysfunction, and cardiovascular disease (CVD) remains to be elucidated.

Vascular endothelial function has been documented to be related to both CVD risk and incidence. Consequently, assessments of flow-mediated dilation (FMD), a measure of vascular endothelial function, and pulse wave velocity (PWV), a measure of vascular stiffness, have grown in popularity as independent predictors of CVD risk. Using the FMD technique, previous studies have demonstrated that patients with COPD display reduced vascular function compared with age-matched controls. Similarly, studies have revealed that patients with COPD exhibit significantly elevated vascular stiffness as assessed by PWV. In addition, there is a growing hypothesis that pulmonary vascular dysfunction itself may be a key factor that instigates the development of COPD and as a consequence systemic vascular dysfunction follows, although the nature of this relationship is currently not well understood.

Chronic inflammation associated with COPD may be the instigator of, and related to, the peripheral vascular dysfunction associated with this population. However, the
downstream effects of this inflammation and the subsequent role of free radicals in disrupting vascular function in these patients have received little attention. To date, we are unaware of a single study that has aimed to reduce free radicals in patients with COPD to determine whether this can improve vascular function, thus providing a mechanistic link between oxidative stress and the elevated CVD risk, incidence, and mortality in this population.

Previously, it has been documented that an acute antioxidant cocktail (AOC) of known efficacy is capable of improving vascular function, as assessed by FMD, in the elderly population and heart transplant recipients with history of heart failure. These previous studies highlight the role of nitric oxide (NO) bioavailability, thus increasing endothelial balance likely attenuates free radical–mediated reductions in incident, respectively. The AOC-induced improvement in redox balance likely attenuates free radical–mediated reductions in nitric oxide (NO) bioavailability, thus increasing endothelial-dependent FMD. These previous studies highlight the role of free radicals in mediating vascular dysfunction in vulnerable populations and that the AOC-induced improvements in vascular function were mediated by an improvement in redox balance.

Accordingly, by acutely administering an AOC to patients with COPD, we sought to determine the role of redox balance in vascular dysfunction (FMD and PWV) in patients with COPD. Specifically, we used FMD and PWV to assess vascular endothelial function and stiffness after acute ingestion of an oral AOC or placebo in patients with COPD and age-matched controls. We hypothesized that the AOC would improve FMD and reduce PWV in patients with COPD but not in controls, highlighting the role of redox balance in vascular dysfunction in this patient population.

Methods

Subjects and General Procedures

Sixty volunteers were recruited for this study: 30 patients with COPD and 30 age- and sex-matched controls (Table 1). Although the majority of patients with COPD had a significant or recent history of smoking (months since quitting: 96±33), current smokers were excluded because acute smoke inhalation is capable of altering endothelial function. A single control subject reported a history of smoking but quit 240 months before the study. In accordance with recent guidelines, the inclusion criterion for patients with COPD was a pulmonary function test that was performed after bronchodilator administration, indicating a forced expiratory volume/fixed vital capacity ratio <0.70. In addition, none of the patients with COPD reported a recent exacerbation (<3 months; Table 2) and were stable, in terms of symptom severity, during both visits. Subject characteristics, such as prevalence of coronary artery disease and obstructive sleep apnea, unless otherwise indicated, were determined from health histories. The protocol was approved by the Institutional Review Boards of the University of Utah and the Salt Lake City Department of Veterans Affairs Medical Center. Written informed consent was obtained from each subject before participation in this study.

All subjects reported to the laboratory twice within 1 week (>48 hours apart) after ingesting either placebo or the AOC in a balanced, double-blind, crossover design. The standardized AOC, taken in the same manner, by all subjects was composed of 2 separate doses of vitamin C, vitamin E, and α-lipoic acid, which has previously been documented to reduce plasma free radicals as measured by electron paramagnetic resonance (EPR) spectroscopy. The first AOC dose (300 mg α-lipoic acid, 500 mg vitamin C, 200 IU vitamin E) was taken 90 minutes before testing, whereas the second AOC dose (300 mg α-lipoic acid, 500 mg vitamin C, 400 IU vitamin E) was taken 60 minutes before testing. These doses and the dosing paradigm were chosen based on both practicality (doses found in over-the-counter formulations) and efficacy as assessed by a reduction in the free radical EPR signal. Placebo microcrystalline cellulose capsules of similar taste, color, and appearance were likewise consumed in the same manner as the AOC trial. Subjects reported to the laboratory in a fasted state and without caffeine or alcohol use for 12 and 24 hours, respectively. They also had not performed any exercise within the past 24 hours. On arrival, a venous blood sample was obtained for blood chemistry (electrolytes, creatinine, glucose, and so on), lipid panel, complete blood count, and biochemical assays (markers of antioxidant capacity and oxidative stress). After this blood draw, subjects were positioned supine and rested quietly for 20 minutes before PWV and FMD testing.

Brachial Artery FMD and Reactive Hyperemia

The FMD was performed in accordance with recent guidelines. Briefly, after baseline measurements of brachial artery diameter and

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n=30)</th>
<th>COPD (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>66±2</td>
<td>66±2</td>
</tr>
<tr>
<td>Female/male, n</td>
<td>15/15</td>
<td>15/15</td>
</tr>
<tr>
<td>Height, cm</td>
<td>169±2</td>
<td>166±2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74±3</td>
<td>73±4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25±1</td>
<td>26±1</td>
</tr>
<tr>
<td>SPB, mm Hg</td>
<td>129±3</td>
<td>136±4</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>79±1</td>
<td>80±2</td>
</tr>
<tr>
<td>FEV₁.0 - L</td>
<td>3.0±0.2</td>
<td>1.3±0.1*</td>
</tr>
<tr>
<td>FEV₁.0 % predicted</td>
<td>107±4</td>
<td>55±4*</td>
</tr>
<tr>
<td>FVC, L</td>
<td>4.0±0.3</td>
<td>3.0±0.2*</td>
</tr>
<tr>
<td>FEV₁.0/FVC ratio, %</td>
<td>76±1</td>
<td>45±3*</td>
</tr>
<tr>
<td>GOLD classification, (n/group)</td>
<td>Mild</td>
<td>3</td>
</tr>
<tr>
<td>Moderate</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Very severe</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.0±0.0</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Urea nitrogen, mg/dL</td>
<td>16.3±0.6</td>
<td>16±1.2</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>85±3.8</td>
<td>87±3.2</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>196±10</td>
<td>195±11</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>51±3.2</td>
<td>57±4.1</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>128±8</td>
<td>121±9</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>144±19</td>
<td>110±12</td>
</tr>
<tr>
<td>Erythrocytes, K/μL</td>
<td>5.1±0.1</td>
<td>5.0±0.1</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>15.2±0.3</td>
<td>15.4±0.5</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>45±0.7</td>
<td>47±1.5</td>
</tr>
<tr>
<td>Leukocytes, K/μL</td>
<td>5.6±0.3</td>
<td>7.4±0.6*</td>
</tr>
<tr>
<td>Neutrophils, K/μL</td>
<td>3.4±0.3</td>
<td>4.8±0.5*</td>
</tr>
<tr>
<td>Lymphocytes, K/μL</td>
<td>1.6±0.1</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>Monocytes, K/μL</td>
<td>0.5±0.0</td>
<td>0.6±0.0*</td>
</tr>
<tr>
<td>Eosinophils, K/μL</td>
<td>0.3±0.1</td>
<td>0.2±0.0</td>
</tr>
<tr>
<td>Basophils, K/μL</td>
<td>0.1±0.0</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE. BMI indicates body mass index; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; FEV₁, forced expiratory volume; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and SBP, systolic blood pressure. *P<0.05 control vs COPD.
Ives et al  Vascular Dysfunction in COPD and Redox Balance

Table 2. Subject History of Controls (n=30) and Patients With COPD (n=30)

<table>
<thead>
<tr>
<th>History</th>
<th>Control (No. of Cases)</th>
<th>COPD (No. of Cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>7</td>
<td>17*</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Chronic heart failure</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Anemia</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Self-reported tobacco smoking</td>
<td>1</td>
<td>26*</td>
</tr>
<tr>
<td>Obstructive sleep apnea</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Exacerbation in past 3 mo req hospitalization</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Exacerbation in past 6 mo req corticosteroids</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Prescribed supplemental oxygen</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Taking supplemental oxygen</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short-acting anticholinergic bronchodilators</td>
<td>0</td>
<td>5*</td>
</tr>
<tr>
<td>Long-acting anticholinergic bronchodilators</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Long-acting β2 agonist</td>
<td>1</td>
<td>14*</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>0</td>
<td>11*</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium channel inhibitor</td>
<td>2</td>
<td>9*</td>
</tr>
<tr>
<td>Antiarhythmic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Thiazide diuretic</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>2</td>
<td>10*</td>
</tr>
<tr>
<td>Angiotensin receptor blocker</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Statin</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Anticoagulant</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

n=30 controls and n=30 patients with COPD. ACE indicates angiotensin-converting enzyme; COPD, chronic obstructive pulmonary disease; and req, requiring.

*P<0.05 control vs patients with COPD.

blood velocity were performed, a blood pressure cuff was inflated to a suprasystolic pressure for 5 minutes. On cuff release, measurements were assessed continuously for 2 minutes. During baseline and cuff release, images were sent in real-time to off-line software and later analyzed using automated edge detection. These analyses were performed by a trained technician who was blinded to both subject group and condition. FMD was quantified as the peak diameter observed postocclusion and expressed as percent change from baseline (%). Reactive hyperemia (RH) was quantified as the cumulative area under the curve (AUC) for brachial artery blood flow during the entire 2-minute postocclusion period.

Pulse Wave Velocity

Concurrent pilot work in a separate study revealed a positive effect of the AOC on PWV in patients with COPD; therefore, because of the timing of this observation, this measurement was only performed in patients with COPD (n=17) and age- and sex-matched controls (n=17) during the latter portion of the current study. Before the FMD test, ultrasound Doppler measurements were taken at the carotid and radial arteries to assess peripheral arterial stiffness (carotid-radial PWV), an approach previously used in this population42 and has been documented to detect elevations in PWV in populations with heightened CVD risk.31,42 In addition, in young healthy individuals, we have found that carotid-radial and carotid-femoral PWV are significantly related, and carotid-radial PWV is, therefore, a predictor of carotid-femoral PWV (r=0.5; S. Ives, PhD, unpublished observations, 2013). PWV was calculated using the foot-to-foot ECG-gated method as described previously43,44 and expressed as meters per second (m·s−1).

Data Analysis

AUC for shear rate and RH was calculated using the trapezoidal rule for the 2 minutes after cuff deflation. Statistical comparisons were performed using 2-way ANOVA, ANCOVA, t tests, and χ², where appropriate. The level of significance was established at 0.05. All data are expressed as mean±SEM.

Results

Subject Characteristics

The subject characteristics and medical history are presented in Tables 1 and 2, respectively. The subjects were well matched, aside from the typical greater incidence of hypertension in patients with COPD, compared with controls. Of note, because there was no difference in vascular function (FMD) between patients with COPD who had a history of hypertension and those who did not (P=0.56), the patients were not divided into 2 groups based on this characteristic. There were no sex differences with regard to basal vascular function (FMD or PWV) in either group or in response to the AOC; therefore, the data for both sexes were combined.

FMD and RH

Under the placebo condition, patients with COPD displayed significantly lower FMD compared with controls (3.1±0.5 versus 6.7±0.6%; P<0.05; COPD versus control; Figure 1). FMD was significantly improved with ingestion of AOC in patients with COPD (3.1±0.5 versus 4.7±0.6%; P<0.05; placebo versus AOC) but not in controls (6.7±0.6 versus 6.9±0.7%; P>0.05; placebo versus AOC; Figure 1). These results also held true when expressed as absolute change in brachial artery diameter, confirming a lower FMD in patients with COPD under placebo condition (0.01±0.002 versus 0.03±0.003 cm; P<0.05; COPD versus control) that was improved by ingestion of the AOC in patients (0.01±0.002 versus 0.02±0.003 cm; P<0.05; placebo versus AOC) and not in controls (0.03±0.003 versus 0.03±0.004 cm; P>0.05; placebo versus AOC). Shear rate (AUC) was not different between patients with COPD and controls (28.77±6.3305 versus 31.56±2.7977 s−1; P>0.05; COPD versus control), and the AOC had no significant effect on shear rate in either group (COPD: 31.308±3.586; control: 31.902±3.034 s−1; P>0.05). Similarly, RH (AUC) was not different between groups (516±68 versus 590±65 mL; P>0.05;
COPD versus control, respectively), and the AOC had no effect on the RH (AUC) in either group (56±75 versus 58±68 mL; \(P<0.05\); COPD versus control, respectively).

To account for potential individual differences in shear rate and thus the stimulus for FMD, the FMD data were normalized for the shear stimulus (FMD/shear rate, AUC). Patients with COPD again exhibited reduced vascular function (0.12±0.03 versus 0.25±0.03 FMD/shear rate; \(P<0.05\); COPD versus control), which was significantly improved after acute ingestion of the oral AOC in patients with COPD (\(P<0.05\)) but not in controls (0.26±0.06 versus 0.28±0.04 FMD/shear rate; \(P>0.05\); COPD versus control). Time to peak vasodilation did not differ between control and patients with COPD and was unaffected by the AOC in both groups.

Post hoc analysis of the subject characteristics revealed a significantly greater incidence of hypertension in patients with COPD. With the potential that this specific comorbidity in patients with COPD could have influenced their vascular function, the data were reanalyzed in 3 different ways to determine whether hypertension, per se, played a role. In the first approach, in addition to age and sex matching, the groups were matched for incidence of hypertension (dropping 10 patients with hypertension in the COPD group and 10 patients without hypertension in the healthy group), leaving n=7 hypertensives in each group of 20 subjects (\(R^2=0.013\); partial \(R^2=0.013\); partial \(\eta^2=0.013\); \(P=0.40\)). Regardless of which approach/group of patients with COPD was examined, there was no statistically significant relationship between the indices of COPD severity (Global Initiative for Chronic Obstructive Lung Disease classification, forced expiratory volume/forced vital capacity ratio, % predicted forced expiratory volume, and so on) and AOC-induced vascular function improvement.

**Pulse Wave Velocity**

Under the placebo condition, patients with COPD exhibited significantly higher PWV (14±1 versus 11±2 m·s\(^{-1}\); \(P<0.05\); COPD versus controls, respectively; Figure 2). The AOC significantly reduced PWV in patients with COPD (14±1 versus 11±1 m·s\(^{-1}\); \(P<0.05\); control versus AOC, respectively) but not in controls (11±2 versus 10±1 m·s\(^{-1}\); \(P>0.05\); control versus AOC, respectively).

**Blood Assays**

Analysis of the patients’ blood revealed lower initial levels of vitamin C (10±1 versus 14±1 µg/mL; \(P<0.05\); COPD versus control), whereas the plasma levels of both groups were significantly \((P<0.05); placebo versus AOC\) increased after AOC ingestion, and patients with COPD still exhibited lower levels of vitamin C even after ingestion of the AOC (18±2 versus 24±2 µg/mL; \(P<0.05\); COPD versus control; Figure 3). Global antioxidant capacity assessed using the ferric reducing ability of plasma was similar at baseline (1.2±0.05 versus 1.0±0.05 mmol/L; \(P<0.05\); COPD versus control) and was significantly \((P<0.05); placebo versus AOC\) increased in both groups after AOC ingestion (1.2±0.06 versus 1.1±0.05 mmol/L; \(P<0.05\); COPD versus control, respectively; Figure 3). Superoxide dismutase activity was lower in patients at baseline (5.1±0.2 versus 8.6±0.7 U/mL; \(P<0.05\); COPD versus control) and only significantly increased in patients with COPD after AOC ingestion \((P<0.05)\). Despite this AOC-induced increase, superoxide

---

**Figure 1.** Flow-mediated dilation (FMD), expressed as peak relative change in patients with chronic obstructive pulmonary disease (COPD) and age- and sex-matched controls under placebo and antioxidant conditions. \(^*\) \(P<0.05\) COPD vs control placebo condition, **\(P<0.05\) placebo vs antioxidant in COPD only.

---

**Figure 2.** Carotid-radial pulse wave velocity under placebo and antioxidant conditions in both patients with chronic obstructive pulmonary disease (COPD), and age- and sex-matched controls. \(^*\) \(P<0.05\) COPD vs control placebo condition, **\(P<0.05\) placebo vs antioxidant in COPD only. Dashed line indicates the recommended 12 m·s\(^{-1}\) cutoff, as established by the Reference Values for Arterial Stiffness Collaboration, indicating elevated risk for cardiovascular disease.
dismutase (SOD) activity remained lower in the patients compared with controls (5.8±0.2 versus 9.1±0.6 U/mL; *P<0.05; COPD versus control; Figure 3). Oxidative stress as assessed by thiobarbituric acid reactive substances (TBARS) and lower endogenous antioxidant capacity (ascorbic acid and SOD) in patients with COPD compared with controls. After AOC ingestion, both groups displayed a similar increase in plasma ascorbic acid and total antioxidant capacity, indicating an equivalent initial effect of the AOC. Under these conditions, the differences in FMD and PWV between patients with COPD and controls were mitigated. Therefore, collectively, these data reveal that patients with COPD exhibit an altered redox balance that seems to negatively affect vascular function and stiffness, likely predisposing this patient population to greater risk and incidence of CVD.

**Discussion**

The goal of this study was to determine the role of redox balance in the vascular dysfunction associated with COPD. Patients with COPD did, indeed, display impaired vascular function, as assessed by FMD and PWV, compared with age-matched controls. Blood analyses revealed greater basal oxidative stress (TBARS) and lower endogenous antioxidant capacity (ascorbic acid and SOD) in patients with COPD compared with controls. After AOC ingestion, both groups displayed a similar increase in plasma ascorbic acid and total antioxidant capacity, indicating an equivalent initial effect of the AOC. Under these conditions, the differences in FMD and PWV between patients with COPD and controls were mitigated. Therefore, collectively, these data reveal that patients with COPD exhibit an altered redox balance that seems to negatively affect vascular function and stiffness, likely predisposing this patient population to greater risk and incidence of CVD.

**Baseline Vascular Dysfunction in Patients With COPD**

In agreement with previous work, using current methodologies for the assessment of FMD, this study has demonstrated that patients with COPD are characterized by reduced vascular function, which is likely specific to the endothelium. In support of this contention, although not assessed in the current study, others have determined that there was no difference between controls and patients with COPD in terms of endothelium-independent dilation using sublingual nitroglycerin or intra-arterial infusion of sodium nitroprusside and verapamil. However, it is also important to note that not all agree that impaired endothelium-dependent dilation is an obligatory component of COPD and may actually depend on exacerbation status. Interestingly, another vascular assessment performed in the current study, RH, was not different between patients with COPD and controls or as a result of ingestion of the AOC. RH, which reflects microvascular responsiveness, seems to be mediated through both endothelium-dependent and endothelium-independent mechanisms but also seems to be predictive of CV events. Thus, a differential response between conduit artery (FMD) and microvascular function (RH) is not surprising and is in agreement with previous work, suggesting that RH alone may not be as sensitive as FMD. However, because RH plays an important role as a component of shear rate, the stimulus for FMD, normalizing FMD for the increase in the shear rate, evoked by cuff occlusion and release, can be an important consideration, although normalizing FMD to shear rate had no effect on the interpretation of the current findings.

In support of the current FMD data, revealing attenuated vascular function in patients with COPD, the PWV assessment
in patients compared with controls under the placebo condition. Of note, placebo PWV in the patients was above the recommended 12 m·s⁻¹ as established by the Reference Values for Arterial Stiffness Collaboration, indicating elevated risk for CVD, which was significantly reduced after acute AOC ingestion. Although PWV is traditionally thought of as purely an estimate of vascular stiffness, more recent evidence suggests that PWV may be related to endothelial function, which may explain the AOC-mediated reduction in PWV. Taken together, reduced FMD and elevated PWV provide significant evidence of vascular dysfunction in patients with COPD, which likely contributes to the elevated CVD risk and prevalence in this population.

Mechanisms of Vascular Dysfunction in Patients With COPD

According to the current findings, the mechanisms responsible for vascular dysfunction in patients with COPD seem to be partly mediated by an alteration in the redox balance by an attenuated antioxidant capacity and elevated oxidative stress because the oral AOC improved vascular function only in those with COPD. Interestingly, in contrast to our previous work that focused on aging, in which the elderly exhibited a significant improvement in FMD after ingestion of the AOC, the relatively old subjects in the current study were not affected by the AOC. However, it is important to note that the control group for patients with COPD were, on average, actually half a decade younger than the subjects in the previous study and contained individuals as young as 36 years of age who likely do not benefit from or may even respond negatively to such exogenous antioxidant treatment. In support of this interpretation, the placebo FMD of the control group in the current study was ≈7%, whereas the FMD of the aged group in the article published by Wray et al was 5%; thus, there is less vascular dysfunction in the current control group likely attributable to a relatively younger cohort, albeit still lower vascular function than young individuals. However, more importantly, the current study reveals that, in comparison with age-matched controls, patients with COPD exhibited reduced vascular function, which can be restored after ingestion of the AOC, suggestive of redox imbalance.

Previous work suggests that antioxidant capacity, as measured by Trolox equivalent antioxidant capacity, was reduced in patients with COPD, resulting in greater superoxide levels in the blood. Numerous mechanisms could contribute to this reduced total antioxidant capacity, such as reduced activity of superoxide dismutase, catalase, and glutathione peroxidase. In the current study, basal levels of factors contributing to the antioxidant defense system, including superoxide dismutase activity and vitamin C, were lower in patients with COPD compared with controls (Figure 3). In parallel, TBARS, which estimates lipid peroxidation, a footprint of oxidative stress, was elevated in patients with COPD (Figure 3) and is in agreement with previous literature suggesting that elevated oxidative stress is a characteristic of COPD. Although we did not see an effect of the acute AOC on TBARS in either group, this lack of an effect agrees with previous work. Specifically, such acute treatments may reduce free radicals either through the inhibition of pro-oxidant enzymes (nicotinamide adenine dinucleotide phosphate oxidase or xanthine oxidase) or through direct molecular quenching, resulting in functional changes, but there is a delay in terms of when the downstream effect of the oxidative stress can be detected (eg, TBARS). The EPR data reported here suggest a reduction in free radicals with ingestion of the AOC in patients with COPD, but with such an approach we cannot ascertain all of the potential mechanisms or the exact origin of the free radicals. Future studies might consider the use of an inhaled formulation to better target the likely source of the inflammation and oxidative stress in the lungs. Considering both the antioxidant status and footprint of oxidative stress (TBARS), the patients with COPD seem to have an altered redox state because of a reduced antioxidant and pro-oxidant environment. An oxidant imbalance contributes to greater levels of the free radicals superoxide and peroxynitrite, both potent endogenous competitors to the endothelial NO-vascular smooth muscle pathway. Superoxide binds avidly with NO, reducing NO bioavailability, which increases the levels of peroxynitrite. Subsequently, peroxynitrite, itself, can oxidize tetrahydrobiopterin, an essential cofactor for endothelial NO synthase, leading to the uncoupling of endothelial NO synthase that also reduces levels of NO via reduced production. In vitro evidence suggests that vitamin C and likely other antioxidants are capable of decreasing the oxidation of tetrahydrobiopterin and preventing the uncoupling of endothelial NO synthase. It is likely that the reduced antioxidant status of patients with COPD resulted in greater free radical-mediated reductions in NO bioavailability either directly through the interaction of superoxide with NO or indirectly through oxidation of tetrahydrobiopterin, which contributed to the blunted endothelial-mediated FMD. In support of the contention that elevated free radicals are mediating the vascular dysfunction in patients with COPD, in the current study there was a significant increase in SOD in response to the AOC, likely because of a sparing of SOD, and a parallel reduction in total free radical signal, as measured by EPR in patients with COPD. Similarly, in agreement with the FMD findings, the PWV data indicated a COPD-related elevation in arterial stiffness, which was significantly reduced after ingestion of the AOC to within the values recommended for lower CVD risk (Figure 2). These results highlight that PWV is also likely dependent on free radical/antioxidant redox balance and NO bioavailability, affecting vasomotor tone and ultimately arterial distensibility in patients with COPD. As always, the effect of disease-specific medications on vascular function in a patient population such as this cannot be ruled out as playing a role in these findings.

Perspectives

Patients with COPD in this study had elevated oxidative stress, lower vitamin C levels, and lower SOD activity, each likely contributing to impaired vascular function, as assessed by FMD and PWV. These findings contrast sharply with the age- and sex-matched controls. Also, of note, although not clearly demonstrating altered vascular function compared with other patients, 17 of the patients with COPD compared with 7 of the controls exhibited medically controlled hypertension.
Because the recruitment process was similar across all subjects, this difference highlights the elevated prevalence and risk of CVD among patients with COPD. It was certainly possible that the greater incidence of hypertension in patients with COPD could have explained the differences between the patients and age-matched controls; however, using several different statistical approaches, there was no evidence that this was the case. Therefore, based on the current findings, it seems reasonable to propose that, in an attempt to improve vascular health and reduce CVD risk in patients with COPD, an increase in antioxidant capacity could be targeted perhaps by exogenous antioxidant supplementation or endogenously through exercise training, thereby reducing free radicals and improving endothelial function. Although the success of the clinical trials using antioxidants as an intervention in CVD has been mixed, it is important to note that most trials have used a single antioxidant and not a cocktail containing both water and fat-soluble vitamins as in the current study. The antioxidant-induced improvement in vascular function, assessed by FMD and PWV, observed in the current study is suggestive of a reduction in the risk of CVD associated with COPD; however, a randomized, controlled trial with long-term supplementation is needed to confirm this hypothesis.

**Experimental Considerations**

As with most studies, especially those performed on clinical populations, there are experimental considerations related to this work that need to be discussed. In patients with COPD, there were a disproportionate number of subjects with hypertension compared with controls, which proved not to influence the conclusions of the current study based on post hoc matching of subjects and ANCOVA with hypertension as a covariate. However, this does raise the question of what would be the effect of this AOC on subjects with a primary diagnosis of hypertension, but this is beyond the scope of the current study that focused on COPD. It should also be recognized that there was not a statistically significant relationship between the indices of COPD severity and AOC-induced vascular function improvement, implying that other uncontrolled variables such as occult obstructive sleep apnea, coronary artery disease, and the effect of several COPD-specific medications (eg, long-acting anticholinergics and β2-agonists) may have contributed to the variance in this study. A larger sample size and more proactive assessments of pathologies that do not come to light through health histories would be required to avoid these issues.

**Conclusions**

Patients with COPD exhibited altered redox balance as evidenced by blunted endogenous antioxidant capacity and elevated oxidative stress compared with age- and sex-matched controls. Vascular function, as measured by FMD and PWV, was impaired in patients with COPD compared with controls. After an acute AOC, antioxidant capacity was improved in patients with COPD, which coincided with significant improvements in vascular function but not in controls. These results highlight the role of redox balance in vascular function of patients with COPD, which likely contributes to the disproportionate risk for CVD in this population.


**Novelty and Significance**

**What Is New?**

- This study has revealed that in comparison with healthy age-matched controls, vascular endothelial function is impaired and vascular stiffness elevated in patients with chronic obstructive pulmonary disease. This may help to explain the increased risk and prevalence of cardiovascular disease (CVD) in patients with chronic obstructive pulmonary disease. An acute oral antioxidant cocktail restored vascular function back to that of controls, highlighting the significant role that redox imbalance likely plays in this population.

**What Is Relevant?**

- Vascular endothelial function and stiffness have been related to CVD, such as hypertension, coronary artery disease, or heart failure. Endothelial dysfunction has been demonstrated to precede the development of CVD. Restoring redox balance, via exogenous antioxidants, in patients with chronic obstructive pulmonary disease may reduce CVD-related morbidity and mortality in this population.

**Summary**

Patients with chronic obstructive pulmonary disease exhibit impaired vascular endothelial function and elevated vascular stiffness, which seems to be mediated by a redox imbalance and may help to explain the increased risk of CVD in this population.
Vascular Dysfunction and Chronic Obstructive Pulmonary Disease: The Role of Redox Balance
Stephen J. Ives, Ryan A. Harris, Melissa A.H. Witman, Anette S. Fjeldstad, Ryan S. Garten, John McDaniel, D. Walter Wray and Russell S. Richardson

Hypertension. published online December 9, 2013; Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2013/12/09/HYPERTENSIONAHA.113.02255

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/