Cerebrovascular and ventilatory responses to acute isocapnic hypoxia in healthy aging and lung disease: effect of vitamin C

Sara E. Hartmann,1,5,8 Xavier Waltz,1,5,8 Christine K. Kissel,2,6,8 Lian Szabo,4,8 Brandie L. Walker,4,7,8 Richard Leigh,1,4,7,8 Todd J. Anderson,2,4,6,8 and Marc J. Poulin1,3,5,6,8,9

1Department of Physiology and Pharmacology, University of Calgary, Calgary, Alberta, Canada; 2Department of Cardiac Sciences, University of Calgary, Calgary, Alberta, Canada; 3Department of Clinical Neuroscience University of Calgary, Calgary, Alberta, Canada; 4Department of Medicine, University of Calgary, Calgary, Alberta, Canada; 5Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada; 6Libin Cardiovascular Institute of Alberta, University of Calgary, Calgary, Alberta, Canada; 7Snyder Institute for Chronic Diseases. University of Calgary, Calgary, Alberta, Canada; 8Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada; and 9Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada

Submitted 14 May 2015; accepted in final form 16 June 2015


Acute hypoxia increases cerebral blood flow (CBF) and ventilation (Ve). It is unknown if these responses are impacted with normal aging, or in patients with enhanced oxidative stress, such as (COPD). The purpose of the study was to 1) investigate the effects of aging and COPD on the cerebrovascular and ventilatory responses to acute hypoxia, and 2) to assess the effect of vitamin C on these responses during hypoxia. In 12 Younger, 14 Older, and 12 COPD, we measured peak cerebral blood flow velocity (Vp; index of CBF), and Ve during two 5-min periods of acute isocapnic hypoxia, under conditions of 1) saline-sham; and 2) intravenous vitamin C. Antioxidants [vitamin C, superoxide dismutase (SOD), glutathione peroxidase, and catalase], oxidative stress [malondialdehyde (MDA) and advanced protein oxidation product], and nitric oxide metabolism end products (NOx) were measured in plasma. Following the administration of vitamin C, vitamin C, SOD, catalase, and MDA increased, while NOx decreased. Vp and Ve sensitivity to hypoxia was reduced in Older by ~60% (P < 0.02). COPD patients exhibited similar Vp and Ve responses to Older (P > 0.05). Vitamin C did not have an effect on the hypoxic Ve response but selectively decreased the Vp sensitivity in Younger only. These findings suggest a reduced integrative reflex (i.e., cerebrovascular and ventilatory) during acute hypoxemia in healthy older adults. Vitamin C does not appear to have a large influence on the cerebrovascular or ventilatory responses during acute hypoxia.

Advanced aging reduces basal CBF (2) and reactivity to CO2 (6), suggesting an age-related impairment within the cerebral vessels. It is presently unknown if the cerebrovascular response to hypoxia is impaired with aging. Indeed, collective evidence implicates the balance release of nitric oxide (NO) produced by endothelial NO synthase (eNOS) in the increase in CBF under resting conditions. While the release of NO from endothelial cells is attenuated with aging, this may provide a mechanism by which older individuals have attenuated cerebrovascular responses. Increased reactive oxygen species (ROS) may lead to NO scavenging in the cerebrovascular endothelium, thereby reducing NO bioavailability and vascular dilation. Antioxidant interventions aimed at improving NO bioavailability, through a reduction of ROS, have been successful in restoring vascular homeostasis in the peripheral circulation (43, 51); however, it is uncertain if improvements would be additionally transferred to the cerebral circulation. Patients with chronic obstructive pulmonary disease (COPD) represent a group of older individuals that are subject to high levels of oxidants, chronic and/or intermittent hypoxemia, and ventilatory and cognitive disturbances. As such, these pathological consequences have the potential to further disrupt the cerebrovascular response to hypoxemia.

The hypoxic ventilatory response (HVR) is a coordinated response determined by peripheral chemoreceptor activation, PaO2, PaCO2, and lung function. Although not a universal finding, aging has been associated with a decrease in the HVR in humans (17, 26, 38); however, the specific mechanisms by which this occurs remains unclear. Indeed, a brisk HVR would seem important in patients with lung disease to maintain oxygenation, particularly in situations where they may be exposed to acute hypoxic events. Previous studies have shown both an increase (13) and decrease (15) in the HVR in COPD patients, but how these responses compare to age-related healthy control subjects is unclear. In addition, the cerebrovascular response to hypoxia determines the tissue acidity (H+/ CO2) of the central chemoreceptors, thus contributing to the overall ventilatory response to hypoxia. The glomus cells of the peripheral chemoreceptors appear to be redox sensitive. An in vitro study by Peng and Prabhaker (34) showed that intermittent hypoxia upregulated hypoxia-inducible factor-1α and...
increased ROS, subsequently increasing carotid body sensitivity. Treatment with the ROS scavenger superoxide dismutase reduced the long-term facilitation indicating an enhancement of carotid body activation with ROS. Along these lines, results from our laboratory have previously shown a strong relationship between the systemic generation of ROS and the increase in the HVR, over a 4-day period of intermittent hypoxia (35). Contrary to these findings, however, ROS has been suggested to inhibit the HVR in humans where anesthetics (45, 46) and acetazolamide (44) were used. In these studies, an antioxidant cocktail (α-tocopherol + vitamin C) reversed the depression of HVR, suggesting an overall, inhibitory effect of ROS on the HVR. It does not appear, however, that in healthy younger adults the HVR is improved via antioxidants alone but rather in a depressed state (45). In elderly women, however, vitamin C was shown to enhance the HVR (36). It would therefore seem plausible that COPD patients may improve their HVR following an antioxidant intervention, secondary to increased disease-related ROS.

Currently, we are unaware of any studies that specifically investigated age-related effects on the cerebrovascular response to acute hypoxia in humans. We therefore set out to determine 1) the cerebrovascular and ventilatory responses to acute isocapnic hypoxia in healthy Older adults and in COPD patients, and 2) test the hypothesis that vitamin C would have the greatest benefit in augmenting the ventilatory and cerebrovascular response to hypoxia in COPD patients. Age-related questions were tested by comparing the physiological responses between healthy Younger and Older study participants. To identify any physiological differences with COPD patients, we compared these responses to the Older control group, who were of a similar age.

MATERIALS AND METHODS

Ethical Approval

All participating subjects who met the inclusion/exclusion criteria for the study provided written, informed consent that conformed to the Declaration of Helsinki. Approvals were obtained from the University of Calgary’s Institutional Conjoint Health Research Ethics Board (E-24463) and the Health Protection Branch of Health Canada (9427-U0206-74C) before commencement.

Study Participants

Healthy volunteers between the ages of 20 and 79 yr (Younger: 20-39 yr; Older and COPD: 55–79 yr) were recruited from the community, and COPD patients were recruited from participating outpatient clinics in Calgary. Subjects were excluded from participating in the study for any of the following reasons: current smoker, body mass index ≥35 kg/m², premenopausal status (excluding Younger), chest pain upon physical exertion, surgery or trauma within previous 6 mo, history of myocardial infarction, angina, arrhythmia, valvular heart disease, chronic heart failure, other/additional lung disease or sleep apnea, history of stroke/cerebrovascular disease, diabetes, peripheral arterial disease, history of chronic headaches/migraines, history of sleep blood clots/thrombosis, uncontrolled hypertension, oxygen therapy, or COPD exacerbation within previous 8 wk. Patients were characterized as having smoking-related COPD on the basis of a physician-diagnosis, with a smoking history >10 pack-yr and at least moderate airflow obstruction (FEV₁/FVC <0.70; FVC ≤80% predicted) evident on spirometry.

Experimental Protocol

Study participants in the Older and COPD groups first completed pulmonary function testing to either rule out undiagnosed lung disease (Older) or to confirm and characterize the COPD diagnosis and severity. The main physiological testing session was conducted on a separate day in the Laboratory of Human Cerebrovascular Physiology, University of Calgary. Before testing, subjects were instructed to refrain from the following: eating or drinking (4 h), caffeine (12 h), vigorous exercise (12 h), vitamin supplementation (72 h), blood pressure medication (24 h), and short- and long-acting bronchodilators (12 and 24 h, respectively, in COPD patients).

Measurements and Procedures

To determine the effects of antioxidants on the acute hypoxic responses, all individuals completed a 5-min hypoxic challenge before and after vitamin C administration. Tests were separated by a 45-min wash-out period. Normal saline was infused in the first experiment as a nonactive sham control, and vitamin C was infused in the second test. A “time control” group was included to insure no carry-over effects between the two hypoxic tests. These subjects underwent an identical protocol to the main study, without an intravenous intervention.

Pulmonary volumes and function. Spirometry, measures of lung volumes, and single-breath diffusion capacity (DLCO) were completed in all Older and COPD subjects by a trained respiratory therapist, as per American Thoracic Society guidelines.

Protocol to measure acute responses to hypoxia. The subject’s resting end-tidal partial pressures of O₂ (PETO₂) and CO₂ (PETCO₂) were recorded using dedicated software (Chamber v2.26; University of Oxford Laboratory of Physiology, Oxford, UK) over a period of 10 min with the subject in a supine position, breathing via a fitted face mask that allowed for nose and/or mouth breathing. PO₂ and PCO₂ in the expired air was sampled continuously (100 Hz) using mass spectrometry (AMIS 2000; Innovision, Odense, Denmark) via a fine catheter. Respiratory volumes were measured with a turbine and volume transducer (VMM-400; Instrumentation Associates, Laguna Niguel, CA), and respiratory flow direction and timing were obtained with a pneumotachograph (RSS100-HR; Hans Rudolf, Kansas City, MO).

With the use of dedicated software (BreathM v2.38; University Laboratory of Physiology, Oxford, UK), the technique of dynamic end-tidal forcing was used to precisely control the desired PETCO₂ and PETO₂ values (39). Breath-by-breath oscillations of the inspired partial pressures of O₂, CO₂, and N₂ were controlled via a fast gas mixing system for precise accuracy and stability of the desired end-tidal values.

Following a 5-min lead-in period of isocapnic euoxia (PETCO₂ = +1.5 mmHg above resting values and PETO₂ = 88.0 mmHg), the protocol progressed with an acute stage of isocapnic hypoxia (PETO₂ = 50.0 mmHg and PETCO₂ = +1.5 mmHg) for a further 5 min. This was then followed by a 2-min stage of isocapnic hyperoxia (PETO₂ = 300 mmHg), aimed to quickly reoxygenate subjects (of particular concern were patients).

Measurement of middle cerebral artery blood flow velocity. Peak CBF velocity (V̇p) was continuously measured in the middle cerebral artery (MCA) during the entire protocol using a 2-MHz pulsed Doppler Ultrasound system (TOC2M; Multigon Industries, Yonkers, NY). Specific search techniques were used to locate the MCA, and the signal was optimized by adjustments to the depth, power, and angle of insonation. A fitted headband secured the Doppler probe for the duration of the test. V̇p was used as an index of CBF. Without changes in the power (P) signal acquired from the Doppler system, cross-sectional area is assumed to be constant. Therefore, without a change in P, V̇p is considered to be a reliable index of CBF. A flow index was calculated using the intensity weighted mean (V̇wM) × P, as previously described (39, 40).
Measurement of cardiovascular variables. Heart rate (HR) was monitored and determined from the R-R interval using a three-lead electrocardiogram (Micromon 7142B monitor; Kontron, Keyenes, UK), and finger pulse photoplethysmography (Portapress; TPD Biomedical Instrumentation, Amsterdam, The Netherlands) was used to measure beat-to-beat blood pressure. Arterial oxygen saturation (SpO2) was monitored using finger pulse oximetry (model 3900; Datex-Ohmeda, Louisville, CO). To increase perfusion to the finger, the hand was warmed with an electric heating pad.

Vitamin C administration. An intravenous catheter was inserted in a vein near the antecubital fossa for drug infusion. Vitamin C (ascorbic acid injection; Alveda Pharma) was diluted with normal saline and administered intravenously. Before the start of the experiment, a loading dose of 3 g ascorbic acid (200 mg/min) was administered over 15 min, followed by a continuous maintenance dose (40 mg/min; 13 ml) during the experiment. This dosage of vitamin C has previously been shown to be effective at rapidly increasing plasma ascorbic acid concentrations more than 15-fold (14). Isotonic normal saline (0.9% NaCl) was infused at an identical flow-rate to ascorbic acid.

Biological markers. Venous blood samples were collected at 3 time points: 1) baseline prior to any experimentation (BL); 2) following the saline-hypoxia test at the completion of the wash-out period (SAL); and 3) following the vitamin C-hypoxia test (Vit C). Blood was centrifuged at 4°C and 3,200 rpm, and plasma and serum were aliquoted and stored at −80°C until analysis.

VITAMIN C AND ANTIOXIDANT ACTIVITY. Serum vitamin C concentrations were measured following manufacturer instructions (Ascorbate Assay Kit; Cayman Chemical, Ann Arbor, MI). Determination of the superoxide dismutase (SOD) activity was performed using the method of Beauchamps and Fridovich (7) slightly modified by Oberley and Spitz (31). Plasma glutathione peroxidase (GPX) was determined by the modified method of Paglia and Valentine (33), using hydrogen peroxide as a substrate. GPX was determined by the rate of oxidation of NADPH to NADP⁺ after addition of glutathione reductase, reduced glutathione, and NADPH. Plasma catalase activity was determined by the method of Johansson and Borg (25), using hydrogen peroxide as a substrate and formaldehyde as a standard. Catalase activity was determined by the formation rate of formaldehyde induced by the reaction of methanol and hydrogen peroxide using catalase as enzyme.

MARKERS OF OXIDATIVE STRESS. Concentrations of plasma malondialdehyde (MDA), a marker of lipid peroxidation, were determined as thiobarbituric reactive substances by a modified method of Ohkawa et al. (32). Plasma concentrations of advanced oxidation of protein products (AOPP) was determined using the semiautomated method by Witko-Sarsat (50). AOPP were measured by spectrophotometry and were calibrated with chloramine-T solution that absorbs at 340 nm in the presence of potassium iodide. The absorbance of the reaction mixture was immediately read at 340 nm against a blank containing PBS, potassium iodide and acetic acid. AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalents.

END PRODUCTS OF NO METABOLISM (NOx). Plasma end products of endothelium NO, nitrates, and nitrates, were measured using the methods of Misko et al. (29). The sum of nitrite and nitrate is considered as an index of NO production (21).

Arterial blood gas analysis. In a subset of participants (Younger: n = 5, Older: n = 4; COPD: n = 4), a 3-F arterial catheter (Cook Medical) was inserted into the distal radial artery to obtain blood gas measurements. Arterial blood samples were drawn into a heparinized syringe (BD Preset) at the end of each stage. Blood was processed immediately (ABL800 FLEX, Radiometer, Copenhagen, Denmark).

Data Analysis

Euvoc-isocapnic (Baseline) breath-breath and beat-beat data were calculated as a 1-min average of the last min of the 5-min euvoc-isocapnic period. Since the peak hypoxic response occurs within 2–3 min, a 1-min average around the peak hypoxic cerebrovascular and ventilatory response was used to indicate the response to isocapnic hypoxia. The sensitivity to hypoxia was calculated as the change (in either VE or V̇p) from isocapnic euvoxia to isocapnic hypoxia divided by the change in SpO2. SpO2 provided the most accurate estimate of arterial O2 saturation. Mean blood pressure (MBP) was calculated as (1/3) × systolic blood pressure + (2/3) × diastolic blood pressure. Cerebrovascular resistance (CVR) was calculated as MBP/VR.

Statistics

Comparisons were separated to include 1) “Younger vs. Older” (i.e., aging comparison), and 2) “Older vs. COPD.” Planned comparisons to identify group differences between the ventilatory and cerebrovascular sensitivity to hypoxia were conducted using independent t-tests. Main effects and interactions were determined using a mixed design 2 × 2 repeated-measurers ANOVA (time × grouping) (SPSS Version 20.0; SPSS, Chicago, IL). Dependent t-tests (within group) and independent t-tests (between-group) were applied if a significant F ratio was detected. The Bonferroni correction factor was used in the case of multiple comparisons. All data are presented as means ± SD, and significance is determined at α-level = 0.05.

RESULTS

Study Participants

Thirty-eight subjects (Younger = 12, Older = 14, COPD = 12) completed the study. In addition to these subjects, two older controls had a reduced FEV1/FVC ratio (i.e., <0.70) and were therefore excluded from the study. Cerebrovascular data for one Older control was excluded due to a lack of suitable MCA signal. Physical characteristics and pulmonary function results are summarized in Table 1. Older participants were of similar age and body mass index but had less smoking history, compared with COPD patients. COPD patients had moderate airflow obstruction (mean FEV1 predicted 62 ± 14%), increased lung volumes, and reduced diffusion capacity, all of which are consistent with the physiological impairment seen in this condition (Table 1).

Time Control Group

A separate subgroup (n = 4) was tested to rule out the effect of repeated hypoxic tests within same day. The test-retest reliability coefficient (i.e., test 1 vs. test 2) for VE and V̇p sensitivity was r = 0.81 and r = 0.86 for VE and V̇p sensitivity, respectively. No significant differences were noted (specifically, V̇p and VE) between trials.

Arterial Blood Gases

Thirteen volunteers participating in the study underwent the procedure of arterial blood gas analysis to validate the use of PETO2 during hypoxia. During hypoxia, PETO2 was ~50.0 mmHg in all groups (Younger = 50.2 ± 0.9; Older = 49.7 ± 1.0; COPD = 49.7 ± 0.5 mmHg; P > 0.05). During hypoxia, there was a progressive decline in PaO2 across the age groups, despite a similar PETO2 [Younger = 53.2 ± 2.6; Older = 48.4 ± 3.7; COPD = 46.1 ± 1.2 (Young vs. Older: P = 0.053; Older vs. COPD: P = 0.279)]. During hypoxia, Younger and
**Table 1. Subject characteristics and pulmonary function**

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Younger</th>
<th>Older</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>12/75</td>
<td>14/68</td>
<td>12/48</td>
</tr>
<tr>
<td>Age, yr</td>
<td>30.3 ± 5.5*</td>
<td>68.4 ± 5.1</td>
<td>68.6 ± 8.1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>71.4 ± 15.2</td>
<td>76.4 ± 13.6</td>
<td>71.1 ± 16.0</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.78 ± 0.05*</td>
<td>1.70 ± 0.07</td>
<td>1.64 ± 0.07*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.1 ± 2.6</td>
<td>26.4 ± 3.5</td>
<td>26.3 ± 5.4</td>
</tr>
<tr>
<td>Smoking history, pack yr</td>
<td>0*</td>
<td>6 ± 11</td>
<td>43 ± 16*</td>
</tr>
<tr>
<td>Pulmonary function (%predicted)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁, liter</td>
<td>—</td>
<td>2.98 ± 0.82 (109 ± 11)</td>
<td>1.49 ± 0.33* (62 ± 14)*</td>
</tr>
<tr>
<td>FVC, liter</td>
<td>—</td>
<td>3.80 ± 0.98 (104 ± 8)</td>
<td>3.08 ± 0.81 (94 ± 21)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>—</td>
<td>0.78 ± 0.51 (104 ± 7)</td>
<td>0.50 ± 0.10* (66 ± 14)*</td>
</tr>
<tr>
<td>FRC, liter</td>
<td>—</td>
<td>3.28 ± 0.57 (107 ± 15)</td>
<td>4.17 ± 1.03* (141 ± 24)*</td>
</tr>
<tr>
<td>RV, liter</td>
<td>—</td>
<td>2.21 ± 0.28 (98 ± 14)</td>
<td>3.35 ± 0.80* (154 ± 36)*</td>
</tr>
<tr>
<td>TLC, liter</td>
<td>—</td>
<td>6.18 ± 1.19 (102 ± 7)</td>
<td>6.23 ± 1.33 (114 ± 17)*</td>
</tr>
<tr>
<td>IC/TLC</td>
<td>—</td>
<td>0.46 ± 0.06 (95 ± 12)</td>
<td>0.33 ± 0.05* (73 ± 10)*</td>
</tr>
<tr>
<td>DLCO, ml·min⁻¹·mmHg⁻¹</td>
<td>—</td>
<td>25.1 ± 7.9 (96 ± 17)</td>
<td>16.14 ± 6.39* (68 ±27)*</td>
</tr>
</tbody>
</table>

*Data presented as means ± SD. Values in parenthesis indicate lung function as %predicted. COPD, chronic obstructive pulmonary disease; BMI, body mass index; FEV₁, forced expiratory volume in 1 s (postbronchodilator); FEV₁/FVC: ratio between forced expiratory volume in 1 s to the forced vital capacity (postbronchodilator); FRC, functional residual capacity; RV, residual volume; TLC, total lung capacity; IC/TLC: ratio between inspiratory capacity and total lung volume; DLCO, diffusion capacity of the lung for carbon monoxide. *P < 0.05, compared with Older group.

**Older** had similar SpO₂ (86 and 85%, respectively); however, COPD patients experienced greater arterial desaturation (81%; P < 0.001). The best (noninvasive) indication of the hypoxic stimulus was finger pulse oximetry (SpO₂) (direct measures of SaO₂ vs. SpO₂; r = 0.96). A poor relationship was found between PetO₂ and PaO₂ (r = 0.49). Therefore, hypoxic sensitivity values were calculated based on finger pulse oximetry.

**Cerebro- and cardiovascular responses to isocapnic hypoxia: effect of vitamin C**

**Control experiment (saline).** Cerebro- and cardiovascular responses to acute isocapnic hypoxia are summarized in Table 2. Basal V̇p was significantly reduced with normal aging at Baseline (saline) (Older: 52.5 ± 11.3 vs. Younger: 68.5 ± 18.5 cm/s; P = 0.024) and during hypoxia (Older: 55.8 ± 11.8 vs. Younger: 76.1 ± 23.3 cm/s; P = 0.018). CVR and blood pressure were lower in Younger both at rest and during hypoxia (P < 0.01). The V̇p sensitivity to hypoxia was decreased in Older compared with Younger (0.28 ± 0.38 vs. 0.74 ± 0.58 cm/s per %desaturation, respectively; P = 0.027; Fig. 1A).

Absolute V̇p was similar between COPD and Older at Baseline (51.2 ± 12.3 vs. 52.5 ± 11.3 cm/s, respectively; P = 0.794) and during hypoxia (56.3 ± 12.5 vs. 55.8 ± 11.8 cm/s, respectively; P = 0.919). Similarly, the V̇p sensitivity to hypoxia was not different between COPD and Older (COPD: 0.74 ± 0.48 vs. Older: 0.91 ± 0.63 cm/s per % desaturation; P = 0.824; Fig. 1A). COPD patients had greater HR than Older at baseline (73 ± 10 vs. 61 ± 8 beats/min, respectively; P = 0.004) and during hypoxia (81 ± 11 vs. 69 ± 11 beats/min, respectively; P = 0.018). Blood pressure and CVR were similar between COPD and Older at baseline and during hypoxia (P > 0.05). All subjects combined, V̇p and ẆVM increased from baseline with hypoxia (V̇p: 56.9 ± 16.1 ± 21.2 ± 5.21 cm/s per %desaturation).

**Table 2. Cardio- and cerebrovascular responses to euoxic isocapnia and isocapnic hypoxia in Younger, Older, and COPD before and after vitamin C infusion**

<table>
<thead>
<tr>
<th></th>
<th>Younger</th>
<th></th>
<th>Older</th>
<th></th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Vitamin C</td>
<td>Control</td>
<td>Vitamin C</td>
<td>Control</td>
</tr>
<tr>
<td>Eucapnic isocapnia V̇p, cm/s</td>
<td>68.5 ± 18.5*</td>
<td>71.5 ± 18.8*</td>
<td>51.4 ± 11.1</td>
<td>54.8 ± 11.0</td>
<td>51.2 ± 12.3</td>
</tr>
<tr>
<td>CVR, mmHg·cm⁻¹·s⁻¹</td>
<td>1.3 ± 0.2*</td>
<td>1.3 ± 0.3*</td>
<td>2.1 ± 0.5</td>
<td>1.9 ± 0.4</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>118 ± 9*</td>
<td>119 ± 7*</td>
<td>137 ± 16</td>
<td>138 ± 13</td>
<td>137 ± 9</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>72 ± 5*</td>
<td>74 ± 5*</td>
<td>85 ± 7</td>
<td>81 ± 6</td>
<td>90 ± 8</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>87 ± 5*</td>
<td>89 ± 5*</td>
<td>101 ± 10</td>
<td>100 ± 7</td>
<td>106 ± 7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>65 ± 8</td>
<td>64 ± 10</td>
<td>61 ± 9</td>
<td>62 ± 10</td>
<td>73 ± 10*</td>
</tr>
<tr>
<td>Isocapnic hypoxia V̇p, (cm/s)</td>
<td>76.1 ± 23.3*</td>
<td>75.9 ± 21.8*</td>
<td>54.4 ± 10.8</td>
<td>57.3 ± 12.4</td>
<td>56.3 ± 12.5</td>
</tr>
<tr>
<td>CVR, mmHg·cm⁻¹·s⁻¹</td>
<td>1.2 ± 0.3*</td>
<td>1.3 ± 0.3*</td>
<td>2.1 ± 0.5</td>
<td>1.9 ± 0.4</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>125 ± 12*</td>
<td>124 ± 9*</td>
<td>145 ± 20</td>
<td>145 ± 15</td>
<td>144 ± 11</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>76 ± 10*</td>
<td>77 ± 9*</td>
<td>87 ± 12</td>
<td>86 ± 8</td>
<td>95 ± 8</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>92 ± 10*</td>
<td>93 ± 8*</td>
<td>106 ± 14</td>
<td>105 ± 9</td>
<td>111 ± 9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>79 ± 12</td>
<td>78 ± 15</td>
<td>69 ± 12</td>
<td>72 ± 13</td>
<td>81 ± 11*</td>
</tr>
<tr>
<td>V̇p sensitivity, cm/s per %desaturation</td>
<td>0.7 ± 0.6*</td>
<td>0.4 ± 0.5†</td>
<td>0.3 ± 0.4</td>
<td>0.2 ± 0.3</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

*Data presented as means ± SD. V̇p, peak cerebral blood flow velocity; CVR, cerebrovascular resistance; SBP, systolic blood pressure, DBP, diastolic blood pressure; MBP, mean arterial blood pressure; HR, heart rate. *P < 0.05, compared with Older, between group under the same condition; †P < 0.05, compared with saline, within-group.
The ventilatory sensitivity to hypoxia was not different between COPD and Older (COPD: 0.7 ± 0.4 vs. Older: 0.7 ± 0.4 l/min per %desaturation; \( P = 0.977 \); Fig. 1B). However, \( \text{SpO}_2 \) was significantly less in COPD compared with Older (COPD: 79.9 ± 3.3% vs. Older: 84.9 ± 1.6%; \( P < 0.001 \)), despite similar \( \text{Pr}_{\text{r},2} \) (COPD: 49.8 ± 0.7 vs. Older: 48.8 ± 1.6 mmHg; \( P = 0.126 \)). A significant correlation was observed between COPD severity (according to FEV\(_1\) predicted) and \( \dot{V}_E \) sensitivity to hypoxia, indicating more severe COPD patients had a higher HVR \( (r = -0.84; P = 0.001) \).

**Effect of vitamin C.** Vitamin C did not have an effect on the ventilatory sensitivity to isocapnic hypoxia in the Younger, Older, or COPD cohorts (Fig. 1B) nor on any ventilatory parameters (i.e., frequency, timing, and inspiratory flow; \( P > 0.05 \)), with the exception of reduced tidal volume in Younger during hypoxia \( (P = 0.008; \text{Table 3}) \).

**Biochemical Markers**

Markers of vitamin C and antioxidant enzyme activities are summarized in Fig. 2. Following vitamin C infusion, serum vitamin C concentration significantly increased \( \sim 80\)-fold from baseline conditions \( (P < 0.001) \). Similarly, SOD and catalase activity increased in all groups following vitamin C infusion. GPX was not affected by vitamin C infusion and remained similar across all conditions, in all groups. MDA increased from baseline conditions in all groups, after vitamin C infusion, however, AOPP was not affected \( (\text{Fig. 3}) \). NOx was decreased following vitamin C intervention in Younger and in Older, only \( (\text{Fig. 4}) \).

**DISCUSSION**

**Major Findings**

The main findings of the present study are 1) the cerebrovascular and ventilatory responses to acute isocapnic hypoxia are reduced in healthy aging; 2) compared with Older adults, COPD patients were found to have a preserved cerebrovascular and ventilatory response; and 3) despite a 80-fold increase in vitamin C, neither the cerebrovascular nor ventilatory responses were augmented during acute hypoxia.

The excellent ROS-scavenging properties of the antioxidant vitamin C are widely acknowledged. Proposed mechanisms of actions of vitamin C include 1) stimulation of NOS activity to increase NO synthesis, 2) free radical scavenging of \( \text{O}_2^- \) to increase NO availability, and 3) enhanced recycling of nitrite to reform NO. In the present study, after vitamin C administration, serum levels of vitamin C increased, with acute increases in SOD, across all groups. Our findings are in line with those of Chen et al.\( (10) \), showing that in mice, vitamin C increases SOD activity (and subsequent decreases in \( \text{O}_2^- \)) and lowers activity of the \( \text{O}_2^- \) generator, NADPH oxidase. We suspect that enhanced activity of SOD would lead to increased dismutation of \( \text{O}_2^- \), which further decreases \( \text{O}_2^- \) concentration. We

**Ventilatory Responses to Isocapnic Hypoxia: Effect of Vitamin C**

**Control experiment (saline).** Ventilatory responses to eucapnic and isocapnic hypoxia are summarized in Table 3. Younger had greater Baseline and hypoxic venti-
assessed the NO system by measuring the end products of NO metabolism (NOx), nitrite + nitrate. One important consideration is the occurrence of nitrite recycling to form NO, and that nitrite is a representation of a storage pool of potential NO activity, rather than a steadystate measure of NO itself (28). Ascorbate is one of many factors aiding in the recycling of nitrite back to NO. Furthermore, this pathway is enhanced under conditions of acidosis and hypoxia (12). Surprisingly, Younger subjects had decreased NOx following vitamin C administration. One possibility is that this group had enhanced recycling of the nitrite-NO pathway, effectively increasing NO stores following vitamin C.

However, vitamin C can exhibit both pro- and antioxidant properties, depending on its concentration and the presence of free metal ions. By the Fenton reaction vitamin C can reduce transition metals, leading to increased generation of free radicals. However, only under pathological conditions would transition metals be expected to be free (e.g., tissue injury), and this reaction is less likely to occur in vivo. Pro-oxidative effects could also be speculated by increased levels of SOD, secondary to increased O$_2^-$ generation. SOD serves as an important line of defense against oxygen free radicals and specifically catalyzes superoxide (O$_2^-$) to H$_2$O$_2$. Catalase and GPX further reduce H$_2$O$_2$ to form H$_2$O, thereby effectively eradicating ROS. Despite an increase in SOD and catalase, lipid peroxidation (i.e., MDA) was increased.

**Influence of Aging and COPD on the Cerebrovascular Response to Acute Hypoxia**

To our knowledge, no previous study has directly assessed the effect of aging on the cerebrovascular responses to hypoxia in humans. Similarly, we are aware of only one previous study investigating these responses in COPD patients (8). The traditional dogma describes the pial arterioles as the primary regulatory site of regional CBF. Recent studies suggest disparity of contribution among the cerebral vessels in regulating CBF. During modest hypoxia (SaO$_2$ ~ 80%), CBF is likely regulated within the pial arterioles; however, during severe hypoxia (SaO$_2$ ~ 70%), changes in CBF are at least partially related to dilation of the vertebral artery (48). The present data suggest that healthy aging significantly reduces the hypoxic cerebrovascular dilatory response by ~50%. The cerebrovascular reactivity to isocapnic hypoxia in Younger subjects is similar to previous studies (1, 39). Initial experiments performed in rats showed little difference in CBF with aging during moderate hypoxia (18, 22, 23). However, direct comparison is cautioned, as several important aspects differ, such as a difference in species, duration of hypoxic stimulus, and control of PaCO$_2$. Contrary to these findings, however, reduced pial vessel dilation has been reported in older rats in response to adenosine, a primary factor regulating hypoxic vasodilation (30). Furthermore, adenosine blockade eliminated the age-related differences previously found during severe hypoxia (23). The Vr sensitivity to hypoxia in COPD patients in the present study was comparable to previous literature (8), which also reported a preserved cerebrovascular response in COPD patients to isocapnic hypoxia. In our present study, COPD patients experienced a greater arterial desaturation (80%) compared with controls (85–87%), despite PaCO$_2$ being controlled at 50 mmHg, suggesting either greater Vd/Q mismatch, diffusion limitation, shunting, and/or a right shift in the O$_2$ dissociation curve. The implications for this are important and extend beyond the laboratory to situations commonly encountered in daily life such as traveling to higher altitude or flying.

**Effect of Vitamin C on the Cerebrovascular Regulation of Acute Hypoxia in Aging and COPD**

A well-characterized consequence to aging is impaired vascular endothelial function and, hence, a reduced availability of NO (43). While experimental evidence in various species...
suggests the importance of NO in this response, human studies have produced variable findings. Previously, our laboratory showed that NOS inhibition did not have an overall effect on the cerebrovascular response during isocapnic hypoxia (24), whereas contradictory findings were reported by Van Mil et al. (47). Hypoxia is thought to increase systemic levels of oxidative stress, and the brain and cerebrovascular endothelium have been implicated in contributing to this overall accumulation of free radicals during hypoxia (5, 11). While numerous studies have shown vitamin C to restore vascular endothelial function in older and patient populations, the present study is the first to test the efficacy of this intervention in the cerebral circulation during hypoxia. An unexpected finding in the present study was a reduction of \( V_P \) sensitivity to hypoxia in Younger individuals following vitamin C. It is likely that there is an “absolute” requirement for CBF to maintain adequate \( O_2 \) delivery and metabolism during hypoxia. Although the sensitivity to hypoxia decreased, the absolute blood flow during hypoxia was very comparable. We reason that the decreased sensitivity is rather an artifact of a trend towards a higher baseline \( V_P \) following vitamin C in Younger subjects. A second possibility is that vitamin C exhibited pro-oxidant properties, leading to NO scavenging, thereby reducing the NO availability, thus explaining the reduced NOx in Younger. It is possible that redox signaling varies with age, as others have also reported different adaptations to antioxidant supplementation. Specifically, following oral antioxidant supplementation, vascular dilation was restored in the elderly (11), while younger adults had an impaired response (42). It remains possible a regulatory shift occurs with aging and less overall reliance on NO-mediated dilation. This is consistent with recent findings that showed no effect of vitamin C in enhancing peripheral blood flow in older individuals, despite blunted hypoxic vaso-dilation (37). It is possible that the vascular responses to hypoxia do not solely rely on an intact endothelium, as Headrick and Berne (20) have suggested that adenosine mediates

Fig. 2. A–D: A, plasma markers of vitamin C and antioxidant enzyme activities [(B) catalase]; C, superoxide dismutase (SOD), and D, glutathione peroxidase (GPX)] in Younger, Older, and COPD patients at baseline (BL), saline (SAL), and following vitamin C infusion. Values represent means ± SD.
relaxation by both endothelial-dependent and -independent mechanisms. The fact that vitamin C decreased the sensitivity of the hypoxic V̇/ledgerline response in Younger volunteers was an unexpected finding in the present study.

Influence of Aging and COPD on the Acute HVR

Normal aging is associated with several important physiological adaptations. Nonetheless, relatively little consistent information and consensus are available regarding the impact that healthy aging and disease have on the HVR. Similar to others (26), we found a 50% decrease in the HVR in healthy older subjects. Conversely, a recent large-scale study (combined cross-sectional and longitudinal study design) showed a surprising increase in the HVR with aging (27). When P_{aCO_2} falls with hyperventilation (during hypoxia), the ventilatory sensitivity is only 16% of what would be achieved if P_{aCO_2} was held constant (1). Despite a similarity in the hypoxic stimulus between studies, we believe these vastly different findings are due to the difference in protocols; mainly the control of P_{aCO_2}.

We found that COPD patients had a preserved HVR compared with Older control subjects. Despite the clinical importance of defining the hypoxic response, similar data for comparison are lacking. We would have expected a blunted HVR in COPD patients, secondary to mechanical and ventilatory constraints. Conversely, it could be argued that the HVR in COPD patients would have been increased due to presumed episodes of intermittent hypoxia encountered with exertional activities of daily living (35).

Effect of Vitamin C on the Acute HVR in Aging and COPD

Our findings are in contrast to our hypothesis, which predicted increased peripheral chemoreceptor activity in COPD patients, leading to increased HVR. Putative mechanisms of O_2 sensing involve redox-sensitive signaling processes in type I cells of the carotid body. A reduced O_2 tension inhibits potassium-channel activity, thereby increasing influx of calcium and, hence, increased nerve traffic to the carotid-sinus nerve. The reducing properties of vitamin C on carotid body functioning are presently not clear, as studies have produced mixed findings. Pokorski and Marczak (36) found oral supplementation of vitamin C to increase the HVR by 31% in healthy older women (60–80 yr), suggesting the reducing properties of vitamin C affected the redox-sensitive potassium channels. Conversely, the effect of an antioxidant intervention (vitamin C + E) on HVR in young men only appeared to have an effect under various conditions of ventilatory depression, including anesthetic, and acetazolamide (44–46). Our findings do not support the role of vitamin C in augmenting the peripheral chemoreceptors, such that following vitamin C infusion, the HVR was not increased.

Fig. 3. A and B: plasma markers of oxidative stress [malondialdehyde (MDA; A) and advanced oxidation protein products (AOPP; B)] in Younger, Older, and COPD patients at baseline (BL), saline (SAL), and following vitamin C infusion. Values represent means ± SD.

Fig. 4. Plasma markers of nitric oxide metabolism [nitrate + nitrite (NOx)] in Younger, Older, and COPD patients at baseline (BL), saline (SAL), and following vitamin C infusion. Values represent means ± SD.
Experimental Considerations

Cigarette smoking is a primary factor in contributing to cardiovascular disease. While it is possible that an underlying smoking history contributed to the observed differences between Younger and Older, we doubt this to be the case due to the prolonged period that had elapsed between subjects previously being “active” smokers (24–36 yr). This is supported by evidence that suggests at least partial reversibility in endothelial function following smoking cessation (9), although the effects on ventilation are less certain.

We employed the technique of “dynamic end-tidal forcing” to independently control the desired level of PETO2 and PETCO2. While the target level of hypoxia was predetermined at PETO2 = 50 mmHg for all individuals, we found the actual stimulus (i.e., PaO2 and subsequently, SaO2) to vary between groups, likely depending on the arterial end-tidal Po2 gradient. Sensitivities (for V̇ ̇ and V̇̇̇) were therefore calculated based on SpO2 values, which were found to highly correlated with the true SaO2.

Despite the widely used technique of transcranial Doppler ultrasound to measure CBF velocity, some limitations should be acknowledged. The interpretation of transcranial Doppler measurements as a tool that reflects relative changes in blood flow relies on the assumption that the diameter of the insonated vessel remains constant. While transcranial Doppler does not permit the assessment of changes in diameter per se, one way to overcome this is to use the total power of the reflected Doppler signal (P), an index of cross-sectional area (40). By further considering the entire velocity spectrum (i.e., the intensity weighted mean velocity, V̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̈
Engineering Research Council of Canada (RGPIN-2014-05554); to M. J. Poulin). S. E. Hartmann received support from Dr. Chen Fong Doctoral Scholarship (Hotchkiss Brain Institute) and a CIHR operating grant. T. J. Anderson is a senior scholar of the Alberta Heritage Foundation for Medical Research. R. Leigh is the GSK-CHIR Professor of Inflammatory Disease. M. J. Poulin is the Brenda Strafford Foundation Chair in Alzheimer Research.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


