Increased ventilatory response to hyperoxic-hypercapnia in COPD patients following Vitamin C administration

Online Data Supplement

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Methods

Subjects were instructed to fast for 4 hours prior to testing. All participants completed the main experimental session in the Laboratory of Human Cerebrovascular Physiology, University of Calgary

Inclusion/exclusion criteria. Participants were excluded from participating in the study if they met any of the following criteria: current smoker, BMI > 35 $kg \cdot m^{-2}$, pre-menopausal status (excluding *Young*), heart/chest pain upon physical exertion, surgery or trauma within previous 6 months, history of myocardial infarction, angina, arrhythmia, valve disease, chronic heart failure, additional lung disease other than smoking-related COPD, history of stroke, diabetes, cardiovascular or cerebrovascular disease, history of chronic headaches/migraines, history of blood clots/thrombosis, uncontrolled hypertension, domiciliary oxygen therapy, or a recent COPD exacerbation.

Pulmonary Function. Spirometry (pre- and post- bronchodilator), lung volume measurements and single-breath diffusing capacity (DL_{CO}) were completed by a trained respiratory therapist, as per ATS guidelines [1-3].

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, Canada) was diluted in normal saline, and administered intravenously using the following dosing regimen; before the start of the experiment, a loading dose of 3g ascorbic acid (200mg/min) was administered over 15-minutes, followed by a continuous maintenance dose (40mg/min) during the experiment. This dosage of vitamin C has previously been shown to be effective at acutely increasing plasma ascorbic acid concentrations more than 15 fold [4]. Isotonic normal saline (0.9% NaCl) was infused at an identical flow-rate to ascorbic acid. The total volume infused (Trial 1 + Trial 2) was 176 ml.

Time control group. An additional group (n=4) was included to rule out the “time” effect of repeated (non-randomized) study interventions within the same day. These subjects completed an identical protocol (Trial 1 and Trial 2, separated by 45 minutes) below, without vitamin C infusion, to evaluate the effect of repeated tests on physiological variables. The test-

retest reliability coefficient (i.e., intervention 1 *vs.* intervention 2) for $\dot{V}_E$ and $\bar{V}_P$ sensitivity was r = 0.90 and r = 0.76 for $\dot{V}_E$ and $\bar{V}_P$ sensitivity, respectively. We did not observe any significant differences between interventions.

Measurement of middle cerebral artery blood flow velocity. Peak cerebral blood flow velocity ($\bar{V}_P$) in the middle cerebral artery was measured continuously using a 2-MHz pulsed Doppler Ultrasound system (TOC2M, Multigon Industries, Inc., Yonkers, NY), as previously described [5, 6]. $\bar{V}_P$ was used as a surrogate for cerebral blood flow. The power ($\bar{P}$) signal acquired from the Doppler system is used as an indicator for changes in vessel diameter. Therefore, without a change in $\bar{P}$, $\bar{V}_P$ is considered to be a reliable index of flow.

Measurement of cardiovascular variables. Heart rate was determined from the R-R interval using a 3-lead electrocardiogram (Micromon 7142B monitor; Kontron Keynes, UK) which was continuously monitored and data collected at 1000 Hz. Beat-beat blood pressure was monitored continuously, and data collected at 100 Hz using finger pulse photoplethysmography (Portapress; TPD Biodemical Instrumentation, Amsterdam, The Netherlands). Finger pulse oximetry was used to monitor arterial oxygen saturation (Model 3900; Datex-Ohmeda, Louisville, CO) and data collected at 100 Hz.

Hyperoxic-Hypercapnia Protocol. Resting end-tidal partial pressures of O_2 ($P_{ET}O_2$) and CO_2 ($P_{ET}CO_2$) were measured with subjects in a supine position, breathing room air through a face mask which allowed for nose and/or mouth breathing. Expired PO_2 and PCO_2 was sampled continuously (100Hz) using mass spectrometry (AMIS 2000; Innovision, Odense, Denmark) via a fine catheter, and was collected using dedicated software (Chamber v2.26; University of Oxford Laboratory of Physiology, Oxford, UK) over a period of 10 minutes to collect baseline values. Following a 5 minute lead-in period of isocapnic euoxia ($P_{ET}CO_2$ = +1.5 mmHg above resting values and $P_{ET}O_2$ held constant at 88.0 mmHg), the protocol progressed with 2-minute stage of isocapnic-hyperoxia ($P_{ET}O_2$ = 300 mmHg), followed by a 5 minute period of hyperoxic hypercapnia ($P_{ET}O_2$ = 300 mmHg and $P_{ET}CO_2$ = +8 mmHg). The technique of dynamic end-tidal

forcing [5], was used to control the desired $P_{ET}CO_2$ and $P_{ET}O_2$ values (BreatheM v2.38, University Laboratory of Physiology, Oxford, UK). Respiratory volumes were measured with a turbine and volume transducer (VMM-400; Interface Associates, Laguna Niguel, CA), and respiratory flow direction and timing were obtained with a pneumotachograph (RSS100-HR, Hans Rudolf, Kansas City, MO, USA). Breath-by-breath oscillations of the inspired partial pressures of O_2, CO_2, and N_2 were controlled via a fast gas mixing system for precise accuracy and stability of the desired end-tidal values.

Arterial blood gas analysis. After an Allen's test was performed, the skin at the wrist above the radial artery was infiltrated with Xylocaine 1% for local anesthesia. A 3F radial catheter (Cook Medical) was inserted into the radial artery via standard Seldinger technique in sterile fashion. A pressurized bag with heparinized saline (1000 IU heparin /500ml 0.9% sodium chloride) was used to keep the arterial line open in between blood gas sampling. The arterial line was removed after completion of the hyperoxic-hypercapnic protocol and manual pressure was held for 5 minutes.

We include only a subset of individuals for the arterial blood gas analysis due to the invasiveness of the procedure, and logistics involved. Participants were selected based on the availability of medical personnel, and their willingness to undergo this additional procedure.

Table S1. Resting ventilatory, cardio- and cerebro-vascular variables during air breathing, before and after vitamin C infusion

	***Young* (n=12)**		***Older* (n=15)**		**COPD (n=11)**	
	Saline	**Vitamin C**	**Saline**	**Vitamin C**	**Saline**	**Vitamin C**
Ventilatory						
$P_{ET_{CO_2}}$ (mmHg)	36.6 ± 2.7	36.1 ± 2.2	35.1 ± 2.8	35.1 ± 3.5	33.6 ± 4.1	33.1 ± 3.8
$P_{ET_{O_2}}$ (mmHg)	86.0 ± 4.7	87.7 ± 5.3	88.3 ± 5.1	89.7 ± 6.9	86.5 ± 9.3	89.7 ± 6.9
Sa_{O_2} (%)	96 ± 1	96 ± 1	95 ± 2	96 ± 1	93 ± 2*	94 ± 1*
V_T (L)	0.8 ± 0.2	0.8 ± 0.3	0.9 ± 0.4	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3
Bf (breath·min^{-1})	14 ± 3	15.4 ± 3.6†	12 ± 3	13.3 ± 3.2†	14 ± 4	14 ± 4
V_{TI} / T_I (L·s^{-1})	0.41 ± 0.07	0.44 ± 0.05	0.35 ± 0.07	0.40 ± 0.10†	0.49 ± 0.18*	0.50 ± 0.19
T_I/T_{TOT} (%)	42 ± 3	41 ± 4	40 ± 6	38 ± 4†	34 ± 4	35 ± 6
$\dot{V}_E$ (L·min^{-1})	10.3 ± 1.6*	11.0 ± 1.7	8.8 ± 1.7	9.4 ± 2.1	10.1 ± 3.3	11.0 ± 3.3†
Cardio- and cerebro- vascular						
HR, (beats·min^{-1})	64 ± 8	65 ± 9	61 ± 9	62 ± 9†	71 ± 11*	73 ± 13*
MBP (mmHg)	86 ± 8*	87 ± 6*	98 ± 9	99 ± 7	102 ± 11	107 ± 10*†
$\bar{V}_P$ (cm·s^{-1})	66 ± 16*	68 ± 19*	51 ± 10	52 ± 11	50 ± 10	52 ± 11
CVC (cm·s^{-1}/ mmHg)	0.77 ± 0.16*	0.79 ± 0.27*	0.52 ± 0.13	0.54 ± 0.12	0.50 ± 0.14	0.49 ± 0.11

Abbreviations: $P_{ET_{CO_2}}$: end-tidal partial pressure of carbon dioxide; $P_{ET_{O_2}}$: end-tidal partial pressure of oxygen; Sa_{O_2}: oxygen saturation; V_T: expired tidal volume; B*f*: breathing frequency; V_{TI} / T_I : Mean inspiratory flow = Inspired tidal volume/Duration time of inspiration; T_I / T_{TOT}: Ratio of inspiratory time to total breath time; $\dot{V}_E$: minute ventilation; HR: heart rate; MBP: mean arterial blood pressure; $\bar{V}_P$: peak cerebral blood flow velocity; CVC: cerebrovascular conductance (CVC = $\bar{V}_P$ / MBP). Data presented as means ± SD. *Different from *Older* under the same condition †Different from saline, within-group.

Table S2. Arterial blood gas analysis during conditions of air, euoxic-isocapnia, and hyperoxic-hypercapnia in *Young* (n=5), *Older* (n=4), and COPD (n=4).

	AIR		
	***Young* (n=5)**	***Older* (n=4)**	**COPD (n=4)**
Pa_{O_2} (mmHg)	83.0 ± 5.0	74.5 ± 4.1	80.5 ± 10.9
$P_{ET_{O_2}}$ (mmHg)	84.0 ± 4.7	88.5 ± 4.8	93.8 ± 9.9
$Pa_{O_2} - P_{ET_{O_2}}$ (mmHg)	-1.0 ± 2.9	-14.0 ± 8.1	-13.3 ± 4.6
Pa_{CO_2} (mmHg)	34.0 ± 3.1	38.0 ± 2.7	37.2 ± 4.8
$P_{ET_{CO_2}}$ (mmHg)	35.0 ± 2.3	33.7 ± 3.1	30.6 ± 2.0
$Pa_{CO_2} - P_{ET_{CO_2}}$ (mmHg)	-1.0 ± 1.1	4.3 ± 2.3	3.1 ± 4.0
	EUOXIC-ISOCAPNIA		
	***Young* (n=5)**	***Older* (n=4)**	**COPD (n=4)**
Pa_{O_2} (mmHg)	89.5 ± 3.5	80.4 ± 6.2	76.8 ± 4.0
$P_{ET_{O_2}}$ (mmHg)	88.2 ± 0.4	87.1 ± 1.8	87.3 ± 1.1
$Pa_{O_2} - P_{ET_{O_2}}$ (mmHg)	1.3 ± 3.6	-6.7 ± 6.3	-10.5 ± 3.7
Pa_{CO_2} (mmHg)	35.0 ± 2.0	37.3 ± 2.7	38.1 ± 3.1
$P_{ET_{CO_2}}$ (mmHg)	36.4 ± 3.2	36.1 ± 1.7	32.8 ± 5.1
$Pa_{CO_2} - P_{ET_{CO_2}}$ (mmHg)	-1.4 ± 3.3	1.1 ± 2.3	3.7 ± 2.4
	HYPEROXIC-HYPERCAPNIA		
	***Young* (n=5)**	***Older* (n=4)**	**COPD (n=4)**
Pa_{O_2} (mmHg)	293.0 ± 8.5	271.0 ± 15.6	268.5 ± 15.3
$P_{ET_{O_2}}$ (mmHg)	300.4 ± 1.5	300.0 ± 0.9	300.1 ± 0.6
$Pa_{O_2} - P_{ET_{O_2}}$ (mmHg)	-7.4 ± 8.5	-29.0 ± 16.0	-31.6 ± 15.5
Pa_{CO_2} (mmHg)	41.1 ± 4.2	42.3 ± 1.9	41.4 ± 3.7
$P_{ET_{CO_2}}$ (mmHg)	42.9 ± 3.4	41.3 ± 2.0	38.9 ± 4.7
$Pa_{CO_2} - P_{ET_{CO_2}}$ (mmHg)	-1.8 ± 2.3	1.0 ± 1.2	2.5 ± 2.3

Abbreviations: Pa_{O_2}: pressure of arterial O_2; $P_{ET_{O_2}}$: pressure of end-tidal O_2; Pa_{CO_2}: pressure of arterial CO_2; $P_{ET_{CO_2}}$: pressure of end-tidal CO_2. Values represent mean ± SD.

Figure legends

Figure S1. The ventilatory and cerebrovascular responses to hyperoxic–hypercapnia in (A,C) *Young* (□) and *Older* (●) and (B,D) *Older* and *COPD* (Δ) before (*solid line*) and after vitamin C infusion (*dashed line*). The ventilatory sensitivity to CO_2 for only the COPD group was significantly greater following the vitamin C infusion ($P \leq 0.05$) (B). No significant changes were observed following vitamin C in the cerebrovascular response. Values represent mean data ±SD.

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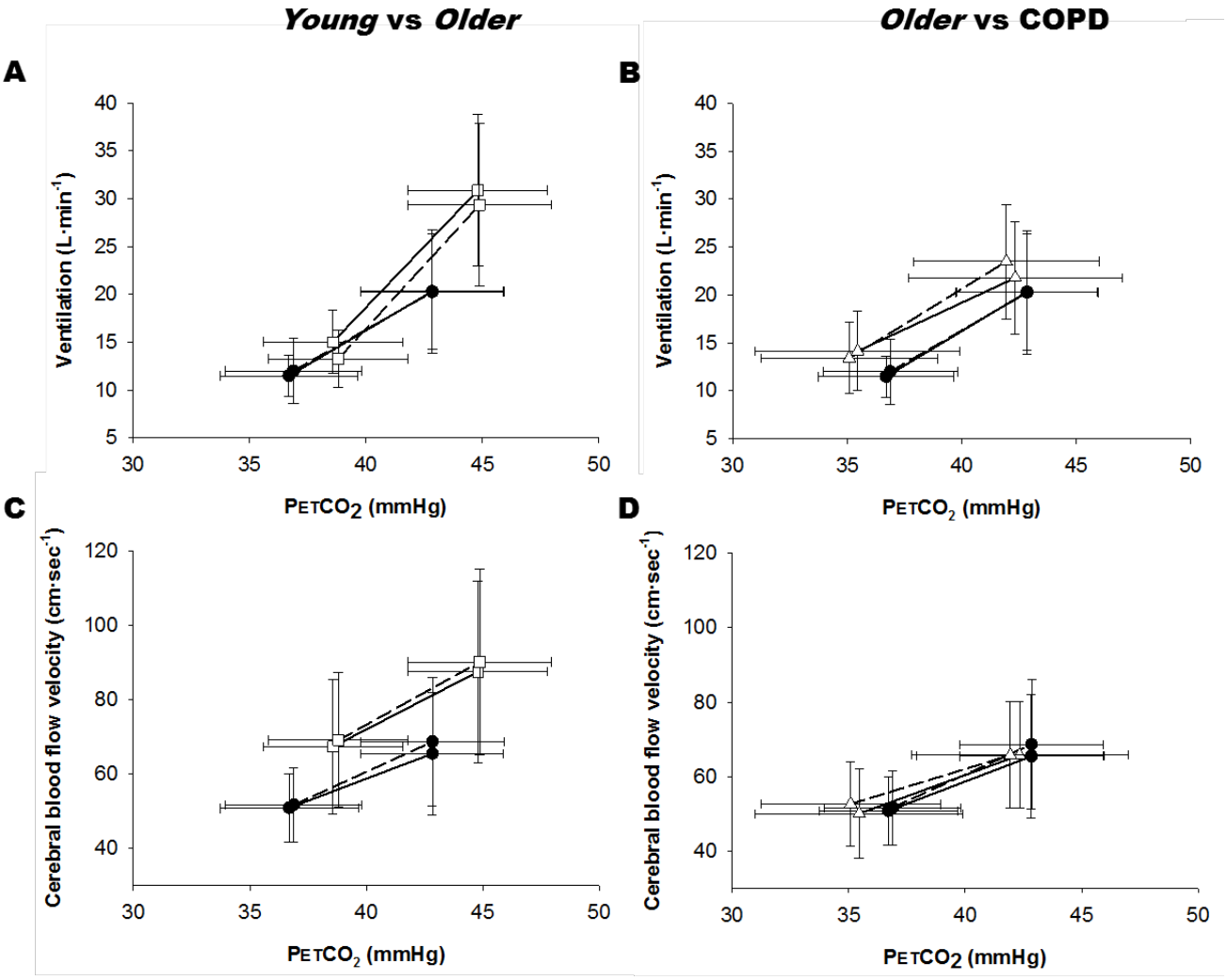
Figure S1.


Young vs Older
Older vs COPD
A
B
C
D
Ventilation (L·min-1)
Cerebral blood flow velocity (cm·sec-1)
PETCO2 (mmHg)
5
10
15
20
25
30
35
40
40
60
80
100
120
30
35
40
45
50