ORIGINAL ARTICLE
COPD

Increased ventilatory response to carbon dioxide in COPD patients following vitamin C administration

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ABSTRACT Patients with chronic obstructive pulmonary disease (COPD) have decreased ventilatory and cerebrovascular responses to hypercapnia. Antioxidants increase the ventilatory response to hypercapnia in healthy humans. Cerebral blood flow is an important determinant of carbon dioxide/hydrogen ion concentration at the central chemoreceptors and may be affected by antioxidants. It is unknown whether antioxidants can improve the ventilatory and cerebral blood flow response in individuals in whom these are diminished. Thus, we aimed to determine the effect of vitamin C administration on the ventilatory and cerebrovascular responses to hypercapnia during healthy ageing and in COPD.

Using transcranial Doppler ultrasound, we measured the ventilatory and cerebral blood flow responses to hyperoxic hypercapnia before and after an intravenous vitamin C infusion in healthy young (*Younger*) and older (*Older*) subjects and in moderate COPD.

Vitamin C increased the ventilatory response in COPD patients (mean (95% CI) 1.1 (0.9–1.1) *versus* 1.5 (1.1–2.0) $L{\cdot}min^{-1}{\cdot}mmHg^{-1}$, $p<0.05$) but not in *Younger* (2.5 (1.9–3.1) *versus* 2.4 (1.9–2.9) $L{\cdot}min^{-1}{\cdot}mmHg^{-1}$, $p>0.05$) or *Older* (1.3 (1.0–1.7) *versus* 1.3 (1.0–1.7) $L{\cdot}min^{-1}{\cdot}mmHg^{-1}$, $p>0.05$) healthy subjects. Vitamin C did not affect the cerebral blood flow response in the young or older healthy subjects or COPD subjects ($p>0.05$).

Vitamin C increases the ventilatory but not cerebrovascular response to hyperoxic hypercapnia in patients with moderate COPD.

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Central chemosensitivity is increased after vitamin C infusion in COPD but not healthy young or older individuals http://ow.ly/Qf2TG

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Introduction

Adequate pulmonary ventilation (V'_E) is essential to effectively regulate carbon dioxide and hydrogen ion concentration, to maintain pH within the normal physiological balance. An increase in arterial carbon dioxide tension (P_{aCO_2}) stimulates the central chemoreflex loop, which leads to an increase in V'_E. This homeostatic mechanism relies on the integration of several processes, including the central chemoreceptors (and to a lesser extent, the peripheral chemoreceptors) located within the brainstem and the effector pathways to the respiratory muscles. While this robust mechanism remains intact in health, aberrations may arise in the presence of respiratory disease, where the chemoreflex loop may be compromised. Chronic obstructive pulmonary disease (COPD) is known to involve many respiratory disturbances, including chemical control, and mechanical impairment. Studies have shown lower ventilatory responses to carbon dioxide in COPD patients [1–4]. While pulmonary constraints alone [5], as observed in COPD, lower the ventilatory response to carbon dioxide, chemosensitivity and respiratory weakness have been suggested to play a role. An initial study from Zakynthinos *et al.* [6] found that oral antioxidant supplementation increased the ventilatory sensitivity to carbon dioxide in healthy adults, suggesting a role for oxidative stress in regulating the sensitivity of the central chemoreceptors. Furthermore, the load (of carbon dioxide) on the central chemoreceptors is largely determined by cerebral blood flow (CBF) due to the proximity of the cerebral vessels to the chemosensitive areas of the brainstem medulla.

The oxidant-scavenging properties of the antioxidant vitamin C have been recognised for decades [7]. Most recently, a high-dose antioxidant cocktail was shown to reduce oxidative stress and restore vascular endothelial function in COPD patients [8]. It is presently unknown whether this same vascular benefit evident in the peripheral vessels is applicable to the cerebral circulation. We and others have previously shown decreased cerebrovascular responsiveness to carbon dioxide in COPD patients [9, 10] and, on this basis, it may thus be possible for vitamin C supplementation to ameliorate this deficit.

The purpose of this study was therefore to evaluate the effect of a vitamin C administration on the ventilatory and cerebrovascular responses to hyperoxic hypercapnia in patients with established, smoking-related COPD of at least moderate severity, compared with a healthy older control group (*Older*). Furthermore, we sought to study the effect of vitamin C on these responses in a healthy young cohort (*Younger*) to establish the effect of ageing, which, to date, remains unknown. We hypothesised that a single infusion of vitamin C would increase the ventilatory response to the greatest extent in COPD patients. Furthermore, we postulated that a blunted cerebrovascular response to hypercapnia in patients with COPD would be normalised following a single vitamin C infusion. This study was part of a larger investigation of vascular and ventilatory responses to a vitamin C administration.

Methods

Subjects

Healthy volunteers between 20 and 80 years were recruited from the community, and patients with established, smoking-related COPD of at least moderate severity (forced expiratory volume in 1 s (FEV_1) <80% of the predicted value and FEV_1/forced vital capacity (FVC) ratio <0.7) were recruited from outpatient clinics in Calgary, AB, Canada. Participants were asked to refrain from the use of short- and long-acting bronchodilators (24 h), blood pressure medication (24 h) and any combination of vitamin–antioxidant supplementation (72 h) before laboratory sessions.

Ethics approval was obtained from the University of Calgary Institutional Conjoint Health Research Ethics Board and the Health Protection Branch of Health Canada (Ottawa, ON, Canada).

Measurements and procedures

COPD subjects and *Older* completed a pulmonary function test. All individuals completed a hypercapnic test 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 an inactive control substance and vitamin C was infused in the second test. The intraclass correlation coefficient for repeated measurements of the ventilatory and cerebrovascular responses to hypercapnia was 0.93 and 0.82, respectively, as determined in a time control group (n=4) that did not undergo vitamin C infusion. Complete methodological details are described in the online supplementary material.

Measurement of vascular variables

Peak cerebral blood flow velocity ($\bar{V}_P$) in the middle cerebral artery (MCA) was continuously measured using transcranial Doppler Ultrasound, as previously described [11, 12]. Heart rate, beat–beat blood pressure and finger pulse oximetry were measured continuously.

Hyperoxic hypercapnia

Testing was conducted in the supine position while breathing through a face mask. End-tidal oxygen (P_{ETO_2}) and carbon dioxide (P_{ETCO_2}) tensions were controlled using the technique of dynamic end-tidal forcing [11] and dedicated software (BreatheM v2.38; University Laboratory of Physiology, Oxford, UK). The protocol progressed as follows: 5 min baseline isocapnic euoxia (P_{ETCO_2} +1.5 mmHg above rest and P_{ETO_2} held at 88.0 mmHg), 2 min isocapnic hyperoxia (P_{ETO_2} 300 mmHg) and 5 min hyperoxic hypercapnia (P_{ETO_2} 300 mmHg and P_{ETCO_2} +8 mmHg). Expired partial pressures oxygen and carbon dioxide were 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; Interface Associates, Laguna Niguel, CA, USA), and respiratory flow direction and timing were obtained with a pneumotachograph (RSS100-HR; Hans Rudolph, Kansas City, MO, USA). Breath-by-breath oscillations of the inspired P_{O_2}, P_{CO_2} and nitrogen tension were controlled *via* a fast gas-mixing system for precise accuracy and stability of the desired end-tidal values.

Vitamin C administration

A 3-g loading dose (200 mg·min^{-1}; 75 mL) of vitamin C (ascorbic acid; Alveda Pharma, Toronto, ON, Canada) was administered intravenously over 15 min followed by a continuous maintenance dose for 24 min (40 mg·min^{-1}; 13 mL) during the experiment. This dosage of vitamin C has previously been shown to be effective at acutely increasing plasma ascorbic acid concentrations >15-fold [13]. Isotonic normal saline (0.9% sodium chloride) was infused at the same flow rate as ascorbic acid.

Arterial blood gas analysis

In a subset of participants (n=13), a catheter was inserted into the radial artery to obtain multiple blood gas measurements. Arterial blood samples were drawn into a pre-heparinised syringe (Preset; BD, Mississauga, ON, Canada) and sampled at the end of each stage. Blood was processed immediately (ABL800 FLEX; Radiometer, Copenhagen, Denmark).

Analysis

The response slopes (*i.e.* V'_E and $\bar{V}_P$) to hypercapnia were determined by linear regression using the last minute of isocapnic hyperoxia and hyperoxic hypercapnia, and relating the change in the dependent variable to the change in P_{ETCO_2}. Mean blood pressure (MBP) was calculated as:

$$\text{MBP} = \frac{1}{3} \times \text{systolic blood pressure} + \frac{2}{3} \times \text{diastolic blood pressure}$$

Cerebrovascular conductance (CVC) was calculated as $\bar{V}_P$/MBP.

The Shapiro–Wilk test was used to assess for normal distribution of the data. For demographic and physiological differences between groups at baseline, data were assessed by comparing 1) *Younger versus Older* and 2) *Older versus* COPD using independent t-tests. Main effects and interactions were determined using a mixed-design 2×2 repeated-measures ANOVA (time×grouping) (SPSS Version 20.0; SPSS Inc., Chicago, IL, USA). Dependent t-tests (within group) and independent t-tests (between groups) were applied if a significant F-ratio was detected. The Bonferroni *post hoc* correction factor was used in the case of multiple comparisons. Data are presented as mean±SD or mean (95% CI) and significance is determined at α-level $\leqslant 0.05$.

Results

Study participants

38 subjects (12 *Younger*, 15 *Older* and 11 COPD) completed the study protocol. One COPD patient could not complete the experiment due to fatigue and breathlessness, and two *Older* had spirometric evidence of mild airflow obstruction (FEV_1/FVC <0.70) and were therefore excluded from the study. Cerebrovascular data for one *Older* were excluded due to a lack of a suitable MCA signal. Physical characteristics and pulmonary function results are shown in table 1.

Effect of vitamin C at rest

Ventilatory variables were not affected by vitamin C at rest in either *Younger* or *Older* or COPD (table 2). *Younger* had higher $\bar{V}_P$ and CVC, and lower MBP than *Older* (table 3). COPD patients had similar $\bar{V}_P$, CVC and MBP, but higher heart rate, compared with *Older* before and after vitamin C. COPD patients had higher diastolic blood pressure only after vitamin C infusion (table 3). Results of the air breathing data and the arterial blood gas analyses are included in the online supplementary material (tables S1 and S2).

TABLE 1 Subject characteristics and pulmonary function data

	Younger	*Older*	COPD
Subjects	12	15	11
Males/females	7/5	7/8	4/7
Age years	30.3±5.5*	68.3±5.0	67.8±8.1
Weight kg	71.4±15.2	75.8±13.3	70.6±16.6
Height, m	1.78±0.05*	1.70±0.07	1.64±0.07*
BMI kg·m^{-2}	24.1±2.6	26.1±3.5	26.3±5.6
Smoking history pack-years	0	13.3±12.9	44.2±16.9*
Pulmonary function			
FEV_1 L (% predicted)		2.98±0.82 (109±11)	1.52±0.33* (63±15)*
FEV_1/FVC (% predicted)		0.78±0.51 (104±7)	0.49±0.10* (65±13)*
FRC L (% predicted)		3.38±0.65 (107±15)	4.25±1.04* (143±24)*
RV L (% predicted)		2.27±0.36 (99 ±15)	3.38±0.83* (156±36)*
TLC L (% predicted)		6.18±1.19 (102±7)	6.35±1.33 (116±17)*
IC/TLC (% predicted)		0.45±0.06 (95±12)	0.33±0.05* (73±11)*
D_{LCO} mL·min^{-1}·mmHg^{-1} (% predicted)		25.2±7.6 (95±17)	16.3±6.7* (69±28)*

Data are presented as n or mean±SD. *Younger*: younger healthy subjects; *Older*: older healthy subjects; COPD: chronic obstructive pulmonary disease; BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; FRC: functional residual capacity; RV: residual volume; TLC: total lung capacity; IC: inspiratory capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide. *: p≤0.05 *versus Older*.

Effect of vitamin C during hyperoxic hypercapnia

Ventilatory

The V'_E response slope was significantly greater in *Younger* than in *Older* but was not altered by vitamin C (figure 1). COPD patients did not have a significantly different V'_E carbon dioxide response to *Older* during the saline control. Following vitamin C, the V'_E response slope significantly increased in COPD patients only (p<0.05) (figures 1 and S1). Contrary to what was observed in *Older*, inspiratory flow and breathing frequency were increased in COPD following vitamin C (table 2).

TABLE 2 Ventilatory responses to euoxic isocapnia and hyperoxic hypercapnia in young healthy (*Younger*), older healthy (*Older*) and chronic obstructive pulmonary disease (COPD) subjects

	Younger		*Older*		COPD	
	Saline	Vitamin C	Saline	Vitamin C	Saline	Vitamin C
Euoxic isocapnia						
P_{ETCO_2} mmHg	38.6±3.0	38.8±3.0	36.7±3.0	36.9±2.9	35.3±5.2	35.1±4.2
S_{aO_2} %	97±1	97±1	96±1	96±1	94±1	94±1
V_T/t_I L·s^{-1}	0.57±0.13*	0.58±0.11*	0.46±0.08	0.45±0.13	0.66±0.12*	0.67±0.18*
t_I/t_{tot} %	42±3	43±3	41±3	42±5	35±3*	35±3*
V_T L per breath	1.1±0.3	1.0±0.2	0.9±0.4	1.0±0.6	1.0±0.3	0.9±0.2
f_R breaths per min	15±4	16±3	14±4	14±4	16±5	16±3
V'_E L·min^{-1}	15.0±3.3*	15.3±3.2*	11.5±2.2	12.0±3.4	14.8±4.6*	14.5±4.4
Hyperoxic hypercapnia						
P_{ETCO_2} mmHg	44.8±3.0	44.9±3.1	42.8±4.0	42.8±3.1	42.3±4.7	41.9±4.0
S_{aO_2} %	99±1	99±1	99±1	99±1	99±1	99±1
V_T/t_I L·s^{-1}	1.05±0.27*	1.01±0.27*	0.75±0.23	0.74±0.19	0.97±0.26*	1.04±0.26*#
t_I/t_{tot} %	46±4*	46±4*	43±3	43±3	36±3*	36±3*
V_T L per breath	1.9±0.4	1.8±0.4	1.6±0.7	1.6±0.7	1.3±0.3	1.3±0.3
f_R breaths per min	17±5	17±5	14±3	14±4	17±4*	19±4*#
V'_E L·min^{-1}	30.9±7.9*	29.4±8.5*	20.3±6.1	20.3±6.5	21.8±5.8	23.5±6.0#

Data are presented as mean±SD. P_{ETCO_2}: end-tidal carbon dioxide tension; S_{aO_2}: arterial oxygen saturation; V_T: tidal volume; t_I: inspiratory time; t_{tot}: total respiratory time; f_R: respiratory frequency; V'_E: minute ventilation. *: p≤0.05 *versus* Older under the same condition; #: p≤0.05 *versus* saline, within group.

TABLE 3 Cardio- and cerebrovascular responses to euoxic isocapnia and hyperoxic hypercapnia in young healthy (*Younger*), older healthy (*Older*) and chronic obstructive pulmonary disease (COPD) subjects

	Younger		*Older*		COPD	
	Saline	**Vitamin C**	**Saline**	**Vitamin C**	**Saline**	**Vitamin C**
Euoxic isocapnia						
$\bar{V}_P$ cm·s^{-1}	67.0±18.1*	69.1±18.1*	50.9±9.2	51.7±9.9	50.1±11.9	52.7±11.2
CVC cm·s^{-1}·mmHg^{-1}	0.74±0.21*	0.78±0.24*	0.50±0.13	0.51±0.12	0.48±0.12	0.48±0.10
SBP mmHg	123±8*	121±9*	140±17	142±14	138±11	144±14
DBP mmHg	73±7*	74±7*	86±10	83±8	91±8	95±11*
MBP mmHg	90±6*	90±6*	104±11	103±8	107±7	111±11
f_C beats per min	64±9	64±9	61±8	63±9	73±11*	75±12*
Hyperoxic hypercapnia						
$\bar{V}_P$ cm·s^{-1}	87.5±24.4*	90.1±25.0*	65.5±16.5	68.7±17.3	65.8±14.3	68.7±14.3
CVC cm·s^{-1}·mmHg^{-1}	0.92±0.24*	0.97±0.30*	0.62±0.18	0.63±0.16	0.57±0.12	0.57±0.11
SBP mmHg	128±10*	125±11*	142±15	150±12	150±14*	157±16*
DBP mmHg	77±8*	78±7*	91±8	90±5	98±8*	102±8*
MBP mmHg	94±7*	94±6*	108±9	110±4	115±9	121±9*
f_C beats per min	67±10	67±10	63±9	65±12	74±10*	76±11*#

Data are presented as mean±SD. $\bar{V}_P$: peak cerebral blood flow velocity; CVC: cerebrovascular conductance (CVC=$\bar{V}_P$/MBP); SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean arterial blood pressure; f_C: cardiac frequency. *: p≤0.5 *versus Older* under the same condition; #: p≤0.05 *versus* saline, within group.

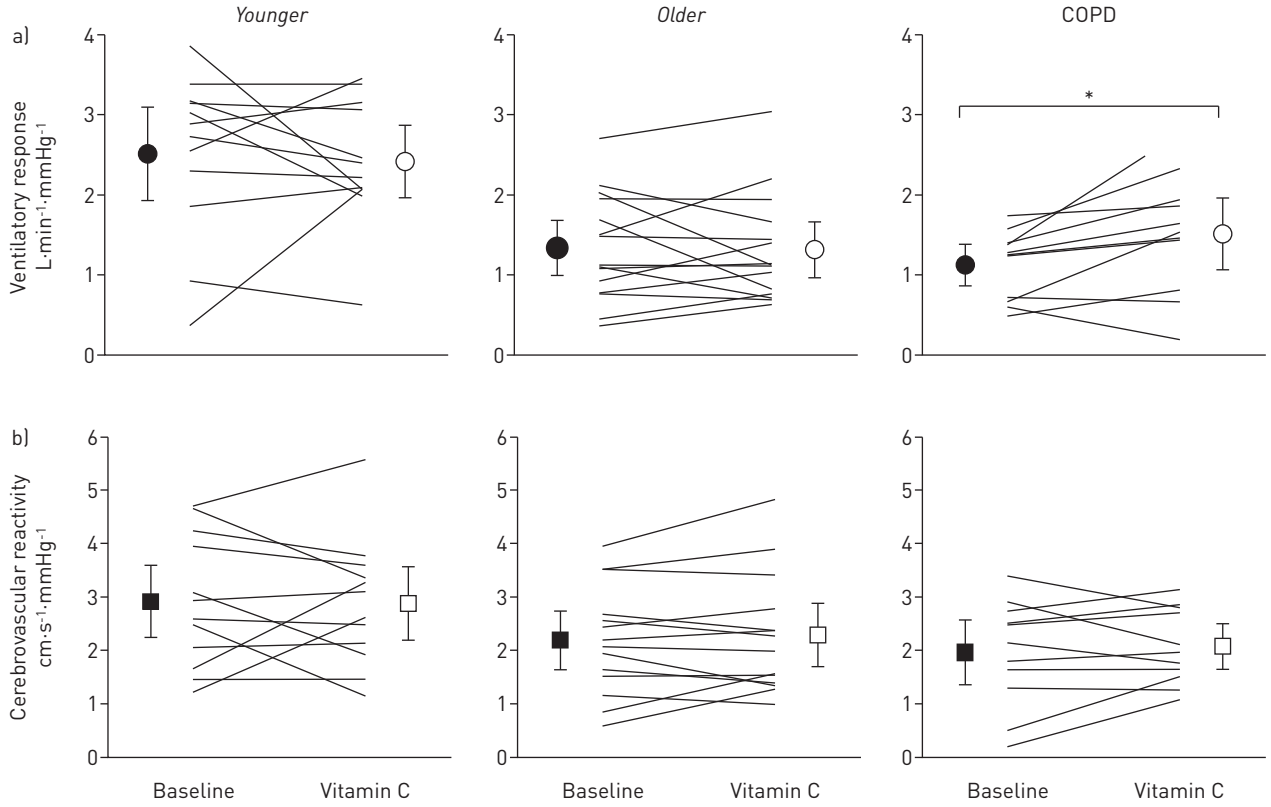

FIGURE 1 Ventilatory and cerebrovascular responses to hyperoxic hypercapnia. Individual (solid lines) and mean values (symbols) for the a) ventilatory (circles) and b) cerebrovascular (squares) responses to hyperoxic hypercapnia in young healthy subjects (*Younger*), older healthy subjects (*Older*) and chronic obstructive pulmonary disease (COPD) patients, at baseline (closed symbols) and with vitamin C (open symbols). Note the increased ventilatory response in COPD patients following vitamin C infusion. Symbols represent group means and whiskers represent 95% confidence intervals. *: p≤0.05 for baseline *versus* vitamin C.

Cerebro- and cardiovascular variables

Absolute $\bar{V}_P$ and CVC were greater in the *Younger* than in *Older* during hypercapnia (table 3). However, the $\bar{V}_P$ reactivity was not different between *Younger* and *Older* under saline conditions (figure 1). Vitamin C did not have an effect on $\bar{V}_P$ reactivity in *Younger* or *Older* (figure 1). $\bar{V}_P$ reactivity did not differ between COPD and the *Older*, and were not different following vitamin C administration (figures 1 and S1).

Disease severity and response to vitamin C infusion

There was a significant negative correlation between FEV_1 (% predicted) and the change in the V'_E carbon dioxide slope observed with vitamin C ($r=-0.53$, $p\leqslant0.001$), indicating that individuals with lower FEV_1 (% predicted) had the greatest increase in the V'_E carbon dioxide response after vitamin C (figure 2). We did not observe any relationship between the change in $\bar{V}_P$ reactivity and FEV_1 following vitamin C (figure 2). No relationship was observed between $\bar{V}_P$ and V'_E response slopes.

Discussion

This is the first study to investigate the role of an antioxidant in the regulation of CBF and the chemical control of breathing in healthy individuals and those with moderate, smoking-related COPD. We found that an acute, intravenous vitamin C administration increased the ventilatory response to hyperoxic hypercapnia in COPD patients but not in healthy young or older subjects. The increase in ventilation was achieved by both an increase in breathing frequency and inspiratory flow. The mechanisms for this observation remain unclear, although they appear to be unrelated to differences in the regulation of CBF.

Ventilatory response to hyperoxic hypercapnia: effect of vitamin C

Perhaps the most striking finding of our study is that vitamin C increased the ventilatory response to carbon dioxide in patients with smoking-related COPD of at least moderate severity, and that the greatest improvements were observed in the most severe patients. In agreement with others [14–16], ageing alone decreased the hypercapnic ventilatory response by approximately 25–50%. Since the ventilatory response to hypercapnia represents an integration of the entire chemoreflex arc (*i.e.* chemosensitivity, neuromechanical coupling and respiratory muscle function), the exact mechanism behind this attenuated response remains elusive. Contrary to the present results, Zakynthinos *et al.* [6], using an oral antioxidant cocktail (vitamins A, C and E, allopurinol, and *N*-acetylcysteine), found an increase in central chemosensitivity as determined by the ventilatory response slope to carbon dioxide in healthy adults. While a cocktail precludes identifying a single mechanism responsible for this change, it also may partially explain the differences observed with the present study. Indeed, this cocktail is effective in reducing oxidative stress during resistive breathing [17]; however, it is not apparent that it was selected specifically to target the central chemoreceptors, despite its effectiveness. Nevertheless, these authors show that a systemic decrease in oxidative stress is sufficient to unload the suspected oxidative stress-induced suppression of central chemoreceptor activity. It is possible that our single-antioxidant intervention was not as effective at reducing whole-body oxidative stress. Indeed, the cellular mechanisms contributing to the increased central chemosensitivity are unclear and results vary between species. Isolated preparations of a rat brain indicate that oxidants stimulate neurons in the solitary complex *via* a decrease in intracellular pH [18]. Vitamin C was selected as an intervention in the present study based on reports

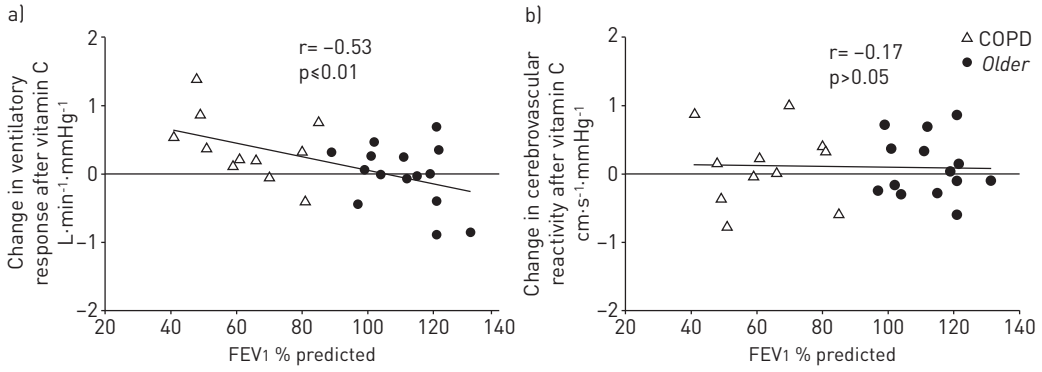

FIGURE 2 Relationship between the change in a) ventilatory and b) cerebrovascular responses in older healthy subjects (*Older*) and chronic obstructive pulmonary disease (COPD) patients during hyperoxic hypercapnia following vitamin C administration, according to lung function. Note the significant relationship between increasing disease severity (forced expiratory volume in 1 s (FEV_1)) and a larger change in ventilatory, but not cerebrovascular, sensitivity after vitamin C infusion.

documenting its ability to acutely decrease systemic oxidative stress and increase nitric oxide bioavailability and restore vascular endothelial function [13, 19, 20].

Nitric oxide may be involved in the control of breathing during hypercapnia. Specifically, neuronal NOS (nNOS) has been implicated in neuromodulation on the ventral surface of the medulla [21]. However, the specific contribution of nitric oxide synthase (NOS) in the ventilatory response to hypercapnia remains undetermined. KLINE and co-workers [22, 23] did not find any difference in the hypercapnic ventilatory response between wild-type and mutant mice (deficient in either endothelial NOS or nNOS), suggesting that NOS has a limited role in the regulation of the ventilatory response to hyperoxic hypercapnia. Conversely, TEPPEMA *et al.* [24] found reduced central and peripheral carbon dioxide sensitivity following systemic NOS blockade in cats.

Our results suggest that 1) suppression of oxidative stress on chemoreceptor activity is greatest in COPD or 2) alternate mechanisms along the chemoreflex arc were affected by vitamin C, such as an improvement in the respiratory muscles, decreased airway resistance or an interaction with the peripheral chemoreceptors. Despite the possibility of reduced diaphragmatic fatigue following vitamin C [25], we doubt this to be a significant contributor, as COPD patients were not ventilatory-limited during the carbon dioxide challenge (~40% maximum voluntary ventilation). The addition of measures of the neural drive to breathe would strengthen our confidence in the interpretation of the ventilatory response to carbon dioxide, particularly when studying a population with ventilatory constraints. Furthermore, although the ventilatory response to carbon dioxide is largely modulated by the central chemoreceptors under conditions of hyperoxia, the contributing role of the peripheral chemoreceptors in modulating this response is open to debate. Indeed, the carotid bodies may play an integral role in determining the respiratory sensitivity of the central chemoreceptors to carbon dioxide [26].

CBF response to hyperoxic hypercapnia: effect of vitamin C

P_{aCO_2} is an important regulator of the cerebral circulation. The dilatory response of the cerebral vasculature in response to increases in P_{aCO_2} is termed "cerebrovascular reactivity" and is often used as an index of cerebrovascular health [27]. The cerebrovascular response to carbon dioxide depends on the availability of endothelial nitric oxide [28]. Alterations in cerebrovascular regulation have been linked to reactive oxygen species-related endothelial dysfunction [29] and/or arterial stiffness [30]. Resting CBF velocity was reduced by ~25% in older compared with young healthy subjects; however, cerebrovascular reactivity was not different with ageing or COPD. Surprisingly, mean arterial blood pressure increased by ~5% in COPD patients (table 3 and table S1). This was not found in either young or older healthy volunteers. Conceivably, we would expect an improvement in vascular endothelial function with vitamin C. It is possible that the intervention affected central cardiovascular function and increased sympathetic outflow. Following vitamin C administration, COPD patients had an increase in heart rate, which may suggest increased cardiac output, contributing to the observed increase in MBP.

While we have previously found women with moderate COPD to have increased systemic oxidative stress and reduced responsiveness to euoxic hypercapnia [9], several possibilities exist to explain the new findings, such as a difference in protocols (euoxic *versus* hyperoxic hypercapnia), anatomical position (supine *versus* upright) and sex differences. It is clear that heterogeneity exists between studies, protocols and disease severity, as others have also reported either a decreased [10] or normal [31] cerebrovascular response to carbon dioxide in COPD patients.

Cerebrovascular reactivity has been found to be restored in patients with elevated cardiovascular risk, secondary to increased nitric oxide availability [32, 33]. Our study reveals that vitamin C does not increase basal CBF or cerebrovascular sensitivity to carbon dioxide. The precise mechanism underlying these findings is not clear at this time. Firstly, it is possible that the brain is well protected against oxidative stress, as the brain has one of the greatest concentrations of vitamin C in the body. Alternate pathways for cerebrodilation may predominate in a state where nitric oxide is compromised (*e.g.* prostaglandins). This is supported by previous findings in our laboratory [34], in which NOS inhibition did not alter the cerebrovascular response to hypercapnia in healthy humans. Secondly, it is also possible that vitamin C does not adequately cross the blood brain barrier, with the preferred method of transport being in the oxidised form, dehydroascorbic acid [35].

Methodological considerations

Because plasma concentrations of vitamin C remain elevated for several hours following intravenous infusion [36], we were unable to perform a randomised control trial of the active and control interventions within the same day, which precludes definitive conclusions from our results. Subjects were not blinded to the intervention, which introduces bias; however, the specific study objects were not revealed. To reduce day–day variability and optimise volunteer retention, the study was completed on the same day. Future

studies performed in a larger cohort that address these limitations would be valuable, particularly to further examine the relationship between disease severity and vitamin C treatment. Secondly, although the plasma concentration of vitamin C is lower in COPD patients than controls and improved with vitamin C supplementation [8], we do not have a measurement of these variables, nor do we have an indication of the antioxidant/oxidative stress balance within the brain. Thirdly, P_{ETCO_2} was used as an estimate of P_{aCO_2}; the accuracy of end-tidal measurements decreases with ageing (and lung disease) as the physiological deadspace increases, resulting in a widening of the P_{ETCO_2}–P_{aCO_2} difference and an underestimation of P_{ETCO_2}. In a subset of volunteers, we validated the end-tidal measurements against arterial measurements and found that during hypercapnia, P_{ETCO_2} was lower than P_{aCO_2} in the COPD and older healthy cohorts but not in young healthy subjects. This has the greatest impact on between-group comparisons but does not affect the interpretation of the effect of vitamin C on the physiological outcomes.

Lastly, transcranial Doppler ultrasound is the most common technique used in humans to measure CBF velocity in the MCA due to its noninvasive properties and high temporal resolution. It is believed that during moderate hypercapnia, the diameter of the MCA does not change, allowing the inference that relative changes in CBF velocity represent similar changes in global CBF [37, 38].

Clinical implications

The role of oxidative stress in COPD and its relationship to cardiovascular disease has received significant attention recently [8]. While we have previously suggested a link between oxidative stress and cerebrovascular function in COPD [9], we were unable to modify the cerebrovascular response with an antioxidant administration in this study. However, our present results do show an involvement of a high-dose antioxidant administration in regulating the ventilatory response to carbon dioxide in COPD. This is an important finding, as a lower ventilatory response experienced in more advanced stages of COPD can lead to greater disturbances in acid–base balance. While our findings implicate a positive role of antioxidants on the ventilatory response, these findings need to be corroborated with future studies that use a more practical dosing regimen that would be available to patients, such as large oral doses.

In conclusion, we found that intravenous vitamin C administration in COPD patients increased the ventilatory, but not cerebrovascular reactivity in COPD patients during hyperoxic hypercapnia. We did not find any influence of vitamin C administration on ventilatory or cerebrovascular sensitivity in healthy young or older healthy volunteers.

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References

1 Scano G, Spinelli A, Duranti R, *et al.* Carbon dioxide responsiveness in COPD patients with and without chronic hypercapnia. *Eur Respir J* 1995; 8: 78–85.
2 Van de Ven M, Colier W, Van der Sluijs M, *et al.* Ventilatory and cerebrovascular responses in normocapnic and hypercapnic COPD patients. *Eur Respir J* 2001; 18: 61–68.
3 Flenley DC, Franklin DH, Millar JS. The hypoxic drive to breathing in chronic bronchitis and emphysema. *Clin Sci* 1970; 38: 503–518.
4 Altose MD, McCauley WC, Kelsen SG, *et al.* Effects of hypercapnia and inspiratory flow-resistive loading on respiratory activity in chronic airways obstruction. *J Clin Invest* 1977; 59: 500–507.
5 Fahey P, Hyde R. "Won't breathe" *vs* "can't breathe". Detection of depressed ventilatory drive in patients with obstructive pulmonary disease. *Chest* 1983; 84: 19–25.
6 Zakynthinos S, Katsaounou P, Karatza M-H, *et al.* Antioxidants increase the ventilatory response to hyperoxic hypercapnia. *Am J Respir Crit Care Med* 2007; 175: 62–68.
7 Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci USA* 1989; 86: 6377–6381.
8 Ives SJ, Harris RA, Witman M, *et al.* Vascular dysfunction and chronic obstructive pulmonary disease: the role of redox balance. *Hypertension* 2014; 63: 459–467.
9 Hartmann SE, Pialoux V, Leigh R, *et al.* Decreased cerebrovascular responses to CO_2 in post-menopausal women with COPD: role of oxidative stress. *Eur Respir J* 2012; 40: 1354–1361.
10 Clivati A, Ciofetti M, Cavestri R, *et al.* Cerebral vascular responsiveness in chronic hypercapnia. *Chest* 1992; 102: 135–138.
11 Poulin MJ, Liang PJ, Robbins PA. Dynamics of the cerebral blood flow response to step changes in end-tidal P_{CO_2} and P_{O_2} in humans. *J Appl Physiol* 1996; 81: 1084–1095.
12 Poulin MJ, Syed RJ, Robbins PA. Assessments of flow by transcranial Doppler ultrasound in the middle cerebral artery during exercise in humans. *J Appl Physiol* 1999; 86: 1632–1637.
13 Eskurza I, Monahan KD, Robinson JA, *et al.* Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. *J Physiol* 2004; 556: 315–324.

14 Brischetto MJ, Millman RP, Peterson DD, *et al.* Effect of aging on ventilatory to exercise and CO_2 response. *J Appl Physiol* 1984; 56: 1143–1150.
15 Peterson D, Pack A, Silage D, *et al.* Effects of aging on ventilatory and occlusion pressure responses to hypoxia and hypercapnia. *Am Rev Respir Dis* 1981; 124: 387–391.
16 Poulin MJ, Cunningham DA, Paterson DH, *et al.* Ventilatory sensitivity to CO_2 in hyperoxia and hypoxia in older aged humans. *J Appl Physiol* 1993; 75: 2209–2216.
17 Vassilakopoulos T, Katsaounou P, Karatza MH, *et al.* Strenuous resistive breathing induces plasma cytokines role of antioxidants and monocytes. *Am J Respir Crit Care Med* 2002; 166: 1572–1578.
18 Mulkey DK, Henderson RA, Ritucci NA, *et al.* Oxidative stress decreases pH_i and Na^+/H^+ exchange and increases excitability of solitary complex neurons from rat brain slices. *Am J Physiol Cell Physiol* 2004; 286: C940–C951.
19 Taddei S, Virdis A, Ghiadoni L, *et al.* Age-related reduction of NO availability and oxidative stress in humans. *Hypertension* 2001; 38: 274–279.
20 Kirby BS, Voyles WF, Simpson CB, *et al.* Endothelium-dependent vasodilatation and exercise hyperaemia in ageing humans: impact of acute ascorbic acid administration. *J Physiol* 2009; 587: 1989–2003.
21 Iadecola C, Faris PL, Hartman BK, *et al.* Localization of NADPH diaphorase in neurons of the rostral ventral medulla: possible role of nitric oxide in central autonomic regulation and oxygen chemoreception. *Brain Res* 1993; 603: 173–179.
22 Kline DD, Yang T, Huang PL, *et al.* Altered respiratory responses to hypoxia in mutant mice deficient in neuronal nitric oxide synthase. *J Physiol* 1998; 511: 273–287.
23 Kline DD, Yang T, Premkumar DR, *et al.* Blunted respiratory responses to hypoxia in mutant mice deficient in nitric oxide synthase-3. *J Appl Physiol* 2000; 88: 1496–1508.
24 Teppema L, Berkenbosch A, Olievier C. Effect of N^{ω}-nitro-L-arginine on ventilatory response to hypercapnia in anesthetized cats. *J Appl Physiol* 1997; 82: 292–297.
25 Supinski G, Nethery D, Stofan D, *et al.* Effect of free radical scavengers on diaphragmatic fatigue. *Am J Respir Crit Care Med* 1997; 155: 622–629.
26 Blain GM, Smith CA, Henderson KS, *et al.* Peripheral chemoreceptors determine the respiratory sensitivity of central chemoreceptors to CO_2. *J Physiol* 2010; 588: 2455–2471.
27 Davenport MH, Hogan DB, Eskes GA, *et al.* Cerebrovascular reserve: the link between fitness and cognitive function? *Exerc Sport Sci Rev* 2012; 40: 153–158.
28 Iadecola C, Pelligrino DA, Moskowitz A, *et al.* Nitric oxide synthase inhibition and cerebrovascular regulation. *J Cereb Blood Flow Metab* 1994; 14: 175–192.
29 Faraci FM. Reactive oxygen species: influence on cerebral vascular tone. *J Appl Physiol* 2006; 100: 739–743.
30 Flück D, Beaudin AE, Steinback CD, *et al.* Effects of aging on the association between cerebrovascular responses to visual stimulation, hypercapnia and arterial stiffness. *Front Physiol* 2014; 5: 49.
31 Bernardi L, Casucci G, Haider T, *et al.* Autonomic and cerebrovascular abnormalities in mild COPD are worsened by chronic smoking. *Eur Respir J* 2008; 32: 1458–1465.
32 Lavi S, Gaitini D, Milloul V, *et al.* Impaired cerebral CO_2 vasoreactivity: association with endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 2006; 291: H1856–H1861.
33 Zimmermann C, Haberl RL. L-Arginine improves diminished cerebral CO_2 reactivity in patients. *Stroke* 2003; 34: 643–647.
34 Ide K, Worthley M, Anderson T, *et al.* Effects of the nitric oxide synthase inhibitor L-NMMA on cerebrovascular and cardiovascular responses to hypoxia and hypercapnia in humans. *J Physiol* 2007; 584: 321–332.
35 Agus DB, Gambhir SS, Pardridge WM, *et al.* Vitamin C crosses the blood-brain barrier in the oxidized form through the glucose transporters. *J Clin Invest* 1997; 100: 2842–2848.
36 Padayatty S, Sun H, Wang Y, *et al.* Vitamin C pharmacokinetics : implications for oral and intravenous use. *Ann Intern Med* 2004; 140: 533–538.
37 Serrador JM, Picot PA, Rutt BK, *et al.* MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 2000; 31: 1672–1678.
38 Giller C, Bowman G, Dyer H, *et al.* Cerebral arterial diameters during changes in blood pressure during craniotomy. *Neurosurgery* 1992; 32: 737–742.