

The carotid chemoreceptor contributes to the elevated arterial stiffness and vasoconstrictor outflow in chronic obstructive pulmonary disease

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Key points

- The reason(s) for the increased central arterial stiffness in chronic obstructive pulmonary disease (COPD) are not well understood.
- In this study, we inhibited the carotid chemoreceptor with both low-dose dopamine and hyperoxia, and observed a decrease in central arterial stiffness and muscle sympathetic nervous activity in COPD patients, while no change was observed in age- and risk-matched controls.
- Carotid chemoreceptor inhibition increased vascular conductance, secondary to reduced arterial blood pressure in COPD patients.
- Findings from the current study suggest that elevated carotid chemoreceptor activity may contribute to the increased arterial stiffness typically observed in COPD patients.

Abstract Chronic obstructive pulmonary disease (COPD) patients have increased central arterial stiffness and muscle sympathetic nervous activity (MSNA), both of which contribute to cardiovascular (CV) dysfunction and increased CV risk. Previous work suggests that COPD patients have elevated carotid chemoreceptor (CC) activity/sensitivity, which may contribute to the elevated MSNA and arterial stiffness. Accordingly, the effect of CC inhibition on central arterial stiffness, MSNA and CV function at rest in COPD patients was examined in a randomized placebo-controlled study. Thirteen mild–moderate COPD patients (forced expired volume in 1 s (FEV₁) predicted \pm SD: $83 \pm 18\%$) and 13 age- and risk-matched controls completed resting CV function measurements with either i.v. saline or i.v. dopamine ($2 \mu\text{g kg}^{-1} \text{min}^{-1}$) while breathing normoxic or hyperoxic air (100% O₂). On a separate day, a subset of COPD

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patients and controls completed MSNA measurements while breathing normoxic or hyperoxic air. Arterial stiffness was determined by pulse-wave velocity (PWV) and MSNA was measured by microneurography. Brachial blood flow was determined using Doppler ultrasound, cardiac output was estimated by impedance cardiography, and vascular conductance was calculated as flow/mean arterial pressure (MAP). CC inhibition with dopamine decreased central and peripheral PWV, and MAP ($P < 0.05$) while increasing vascular conductance in COPD. No change in CV function was observed with dopamine in controls. CC inhibition with hyperoxia decreased peripheral PWV and MSNA ($P < 0.05$) in COPD, while no change was observed in controls. CC inhibition decreased PWV and MSNA, and improved vascular conductance in COPD, suggesting that tonic CC activity is elevated at rest and contributes to the elevated arterial stiffness in COPD.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a respiratory disorder characterized by progressive, partially reversible airway obstruction, dyspnoea and marked exercise intolerance. Although COPD is considered a disease of the lung, there is evidence of substantial systemic manifestations, such as cardiovascular (CV) disease, that develop as a consequence of COPD (Wouters, 2002). Previous work has shown elevated sympathetic nervous activity (SNA) and central arterial stiffness in both hypoxaemic and non-hypoxaemic COPD patients, both of which would contribute to CV deterioration, and are linked to increased CV events/mortality (Sabit *et al.* 2007; Vlachopoulos *et al.* 2010; Andreas *et al.* 2014).

The mechanism(s) causing elevated SNA and central arterial stiffness in COPD are poorly understood; however, recent work suggests that greater activity of the carotid chemoreceptor (CC) may play a role (Stickland *et al.* 2016). The CCs are located within the carotid body at the level of the carotid sinus and are sensitive to circulating stimuli including O_2 , CO_2 , inflammation (IL-6, TNF- α) and reactive oxygen species, and are key mediators of ventilatory control and sympathetic vasoconstrictor outflow (Guyenet, 2000; Ding *et al.* 2011; Porzionato *et al.* 2013). Importantly, the CC has been shown to be tonically active and sensitized in conditions such as chronic heart failure (CHF), leading to elevations in SNA (Sun *et al.* 1999; Marcus *et al.* 2014). Recently we have shown enhanced resting CC activity and sensitivity in non-hypoxaemic non-hypercapnic COPD patients (Stickland *et al.* 2016). CC sensitivity was related to peripheral pulse-wave velocity (PWV), and CC inhibition by breathing 100% O_2 normalized PWV in COPD patients (Stickland *et al.* 2016). These findings are consistent with previous work in hypoxaemic COPD patients with severe respiratory failure demonstrating that breathing 100% O_2 reduced resting SNA (Heindl *et al.* 2001). Combined with

previous work in CHF, these data suggest that the CC may play an important role in CV regulation and may help explain the increased sympathetic outflow and central arterial stiffness even in stable non-hypoxaemic COPD patients. Therefore, the aim of the present study was to examine the effect of CC inhibition on central arterial stiffness and CV function at rest in COPD patients. It was hypothesized that CC inhibition with either 100% inspired O_2 , or intravenous (i.v.) dopamine would reduce central arterial stiffness, secondary to reduced sympathetic vasoconstrictor outflow in COPD patients, while no change would be observed in controls.

Methods

Ethical approval

The present study was a randomized, single-blind, placebo controlled design and was approved by the University of Alberta Health Research Ethics Board (Biomedical Panel Protocol no. 00043106). Participant written informed consent was obtained prior to any research procedures. The study conformed to the standards set by the latest revision of the *Declaration of Helsinki*.

Participants and procedure

Thirteen non-hypoxaemic mild–moderate COPD patients (GOLD Stage I and II, post bronchodilator (forced expired volume in 1 s (FEV₁)/forced vital capacity (FVC) ratio < 0.7 , FEV₁ $\geq 50\%$ predicted) with a smoking history > 10 pack-years and 13 age- and risk-matched controls with minimal smoking history (< 10 pack-years) were recruited. Participants were carefully screened to exclude supplemental oxygen therapy, diabetes, CV disease, body mass index ≥ 35 , severe inflammatory disorders (such as connective tissue disease), severe sleep apnoea (STOP-Bang score < 3 , apnoea–hypopnoea

Table 1. Participant characteristics

	Control	COPD	P value
Participants	13	13	—
Male/female	8/5	10/3	—
Age (years)	64 ± 14	61 ± 14	0.67
Height (cm)	168.7 ± 9.3	170.2 ± 5.5	0.63
Mass (kg)	78.0 ± 15.1	74.4 ± 14.7	0.55
BMI (kg m ⁻²)	27.2 ± 3.7	25.6 ± 3.8	0.26
Smoking history (pack-years)	3.9 ± 4.5	41.8 ± 29.7	<0.001
Current smoker (n)	0	9	—
Modified MRC dyspnoea (scale 0–4)	0.0 ± 0.0	1.3 ± 0.5	<0.001
Medication use (n)			
SABA	0	8	—
ICS	0	8	—
Combined ICS/LABA	0	6	—
β-blocker	1	2	—
ACE-ARB	0	1	—
Diuretics	1	0	—
Statins	4	3	—
Pulmonary function			
FEV ₁ (L)	3.0 ± 0.8	2.4 ± 0.6	<0.001
FEV ₁ (% predicted)	106.9 ± 13.9	83.1 ± 18.0	<0.001
FEV ₁ (Z-score)	0.13 ± 0.86	-1.41 ± 1.26	<0.001
FEV ₁ /FVC (% predicted)	97.2 ± 5.7	79.9 ± 10.1	<0.001
FEV ₁ /FVC (Z-score)	-0.75 ± 0.47	-2.28 ± 1.17	<0.001
FRC (% predicted)	98.4 ± 17.6	118.1 ± 20.5	0.015
RV (% predicted)	87.8 ± 18.9	113.9 ± 21.4	0.004
TLC (% predicted)	100.3 ± 15.3	104.5 ± 15.0	0.555
D _{LCO} (% predicted)	93.4 ± 10.5	65.1 ± 21.6	<0.001
Cardiopulmonary peak exercise responses			
\dot{V}_{O_2} (mL kg ⁻¹ min ⁻¹)	33.3 ± 9.3	24.9 ± 8.9	0.028
\dot{V}_{O_2} (% predicted)	130 ± 25	88 ± 26	0.002
\dot{V}_{CO_2} (L min ⁻¹)	2.83 ± 0.86	1.98 ± 0.71	0.011
\dot{V}_E (L min ⁻¹)	92.6 ± 29.3	68.2 ± 17.3	0.017
P _{ETCO₂} (mmHg)	34.1 ± 4.8	32.8 ± 4.9	0.489
HR (beats min ⁻¹)	162 ± 20	144 ± 22	0.065
S _{pO₂} baseline (%)	95.8 ± 4.2	97.0 ± 1.5	0.412
S _{pO₂} peak (%)	95.6 ± 3.2	95.4 ± 2.5	0.864
Nadir \dot{V}_E/\dot{V}_{CO_2}	29.2 ± 2.2	34.9 ± 5.5	<0.001
Change in IC baseline to peak exercise (L)	0.1 ± 0.4	-0.4 ± 0.4	0.007

Data are presented as *n* or means ± SD. ACE: angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI: body mass index; COPD: chronic obstructive pulmonary disease; D_{LCO}: diffusing capacity of the lung for carbon monoxide; FEV₁: forced expiratory volume in 1 s; FRC: functional residual capacity; FVC: forced vital capacity; HR: heart rate; IC: inspiratory capacity; ICS: inhaled corticosteroid; LABA: long-acting β₂-agonist; MRC: Medical Research Council; P_{ETCO₂}: partial pressure of carbon dioxide; RV: residual volume; SABA: short-acting β-agonist; S_{pO₂}: oxygen saturation measured by pulse oximeter; TLC: total lung capacity; \dot{V}_{CO_2} : carbon dioxide production; \dot{V}_E : minute ventilation; \dot{V}_{O_2} : oxygen uptake. Z-score calculated using Global Lung Initiative 2012 Equations (Quanjer *et al.* 2012).

index < 30 as evaluated by overnight sleep monitoring with ApneaLink Plus, ResMed Ltd, Bella Vista, Australia) and recent respiratory exacerbations (<6 months). Medication use (not including respiratory medication) was similar between the control and COPD groups (Table 1). The lack of CV disease was also confirmed by a normal resting blood pressure (systolic < 140 mmHg) and 12-lead ECG, as well as normal blood pressure and ECG responses to maximal exercise.

Three sessions were completed over a 3 week period in the following order: (1) participant enrolment and medical history, standard pulmonary function and cardiopulmonary exercise test as previously described (Edgell *et al.* 2015; Stickland *et al.* 2016), (2) basal chemoreceptor reflex assessment, and (3) the experimental trial. Prior to all study procedures, participants were asked to abstain from caffeine, smoking, vigorous exercise, alcohol and respiratory medications for at least 6 h preceding testing.

Basal chemoreceptor assessment

Prior to data collection, participants rested quietly in the supine position while breathing normoxic air on a mouthpiece for 10 min to obtain baseline data. Basal CC activity was then evaluated by examining the transient reduction in minute ventilation in response to 2 min of hyperoxia (inspired O₂ fraction, $F_{I_{O_2}} = 1.0$). The difference between the baseline and nadir (15 s) ventilation was used as the index of basal CC activity (Dejours, 1962; Stickland *et al.* 2016). Following a minimum 10 min break, a new baseline ventilation was obtained, and participants then completed a standard hyperoxic hypercapnic rebreath test to determine central medullary chemoreceptor sensitivity (Read, 1967). A 4 L rebreathing bag was filled with a specialized gas mixture (7% CO₂, 50% O₂ and 43% N₂). Following a brief bout of hyperoxic breathing ($F_{I_{O_2}} = 0.5$), participants then began rebreathing until the end-tidal CO₂ values reached 55 mmHg or participants requested to stop. After another minimum 10 min break, baseline ventilation was obtained again and CC sensitivity was evaluated by measuring the hypoxic ventilatory response using a stepwise method, as previously described (Edgell *et al.* 2015; Stickland *et al.* 2016). Briefly, participants breathed normoxic air for 3 min followed by 3 min at a target arterial oxygen saturation ($S_{p_{O_2}}$) of 90% and then 3 min at a target $S_{p_{O_2}}$ of 85%. Arterial saturation was analysed as opposed to end-tidal O₂ due to the uncertainty regarding the accuracy of estimating arterial blood gas from end-tidal expired gas in patients with lung disease (Jones *et al.* 1979). Sufficient rest was given between trials to ensure that ventilation and arterial blood pressure returned to the initial baseline before continuing to the next test.

Experimental trial

Following instrumentation, participants breathed freely on the mouthpiece for 15 min to obtain baseline data. Interventions were then conducted in random order: (1) saline and normoxia, (2) saline and hyperoxia ($F_{I_{O_2}} = 1.0$), (3) dopamine and normoxia, and (4) dopamine and hyperoxia. Each intervention was separated by 10 min. For the dopamine trials, one minute steady-state mean data were recorded following a 10 min wash-in. Two minute hyperoxia interventions were conducted with either saline or dopamine infusion and measurements were analysed in the second minute (Stickland *et al.* 2011). On a separate day, a subset of seven COPD patients and seven controls completed resting muscle SNA (MSNA) measurements while breathing normoxic or hyperoxic air.

Dopamine

Prior to the experimental trial, an i.v. catheter was inserted into the antecubital vein of the left arm. Participants

received either isotonic i.v. saline or i.v. dopamine HCl (2 $\mu\text{g kg}^{-1} \text{min}^{-1}$; Hospira, Lake Forest, IL, USA) via an automated constant-infusion pump (Alaris, San Diego, CA, USA). Dopamine does not cross the blood–brain barrier and would not affect the central chemoreceptors (Zlokovic, 2008). Previous research has shown that this specific dose effectively inhibits the carotid chemoreceptors in humans (Lahiri *et al.* 1980; Stickland *et al.* 2011; Edgell *et al.* 2015).

Hyperoxia

During both the chemoreceptor assessment and the experimental trials, 2 min hyperoxia interventions ($F_{I_{O_2}} = 1.0$) were conducted. Transient hyperoxia has been previously shown to rapidly inhibit the CC (Nye *et al.* 1981). Prolonged bouts of hyperoxia were avoided as long-term hyperoxia can act as a central stimulant, which would confound results from the current study (Dean *et al.* 2004).

Cardiorespiratory measures

For both the chemoreceptor reflex assessment and the experimental trial, all data were recorded and integrated with a data acquisition system (Powerlab 16/30; ADInstruments, New South Wales, Australia) and stored for subsequent analysis using associated software (LabChart 8.0 Pro; ADInstruments). Minute ventilation was measured by a pneumotachometer (3700 series, Hans Rudolph, Kansas City, MO, USA) and expired CO₂ and O₂ were measured (CD-3A and S-3A; AEI Technologies, Naperville, IL, USA) continuously from a small sample port off the mouthpiece to obtain end-tidal CO₂ and O₂. Inspired gas was humidified (HC150; Fisher and Paykel Healthcare, Auckland, New Zealand) and delivered continuously using a flow-through system to prevent rebreathing of expired gas. Inspired gas was modulated using air–oxygen (normoxia to hyperoxia) or air–nitrogen (normoxia to hypoxia) blender systems. Heart rate was determined from the R–R interval with a single lead ECG (lead II, Dual Bio Amp; ADInstruments). Cardiac output was determined with impedance cardiography (Physioflow, Manatec, Paris, France), which has been previously validated (Edmunds *et al.* 1982). Arterial oxygen saturation was estimated with pulse oximetry (N-595; Nellcor Oximax, Boulder, CO, USA) using a left ear-lobe sensor. Blood pressure was determined using finger plethysmography (Finometer Midi, Amsterdam, the Netherlands) and was calibrated to brachial artery pressure at regular intervals using manual auscultation.

Doppler ultrasound

Similar to previous work (Edgell *et al.* 2015), brachial arterial mean blood velocity was obtained from the right

arm using pulse-wave Doppler ultrasound (GE, Vivid-7, 4–5 MHz, <60 deg angle of insonation). Briefly, the average velocity was multiplied by the cross-sectional area of the brachial artery and then multiplied by 60 to determine brachial flow (mL min^{-1}) (Stickland *et al.* 2011; Edgell *et al.* 2015). Brachial conductance was calculated as brachial flow/mean arterial pressure.

Arterial stiffness

Arterial stiffness was determined by PWV. Pulse waves were gathered simultaneously using applanation tonometry (Mikro-tip Catheter Transducers model SPT-301, Millar Instruments, Inc., Houston, TX, USA) from the carotid, radial and femoral arteries. PWV was calculated as $\text{PWV} = D \times \Delta t^{-1}$, where D was the distance (m) between sites and Δt was the time difference (s) between pulse waves using the foot-to-foot method (Laurent *et al.* 2006). Carotid–femoral (cfPWV) and carotid–radial (crPWV) pulse-wave velocity were used as indices of central and peripheral arterial stiffness, respectively (Andreas *et al.* 2014; Stickland *et al.* 2016). Distance was measured on the skin using a tape measure beginning at the sternal notch and extending to the recording sites of the cfPWV and crPWV. At least 10 consecutive beats were averaged to represent PWV over a complete respiratory cycle (Laurent *et al.* 2006).

Muscle sympathetic nerve activity

MSNA was recorded via peroneal microneurography as previously described (Charkoudian *et al.* 2017). Briefly, the raw sympathetic signal was rectified and integrated to produce a neurogram with a characteristic bursting pattern. MSNA was then analysed using a semi-automated peak detection algorithm (Chart 8.1.3; ADInstruments) and expressed as the change in burst frequency (bursts min^{-1}) and incidence (bursts (100 heart beats) $^{-1}$) from baseline to hyperoxia.

Statistical analysis

Data are presented as means \pm standard error of the mean (SEM) unless otherwise stated. For all inferential analysis, statistical significance was set *a priori* at $P < 0.05$. Primary endpoints were carotid–femoral pulse-wave velocity and brachial artery conductance. A multi-factorial ANOVA was used to evaluate the effect of CC inhibition with either dopamine or hyperoxia on key dependent variables in COPD and controls. If a main effect or interaction effect was found, a Bonferroni multiple comparisons test was used to locate the differences. Unpaired t analysis was used to evaluate participant characteristics and chemoreceptor reflexes between groups. All statistical analyses

were performed using Sigma Plot Software version 13 (Systat Software Inc., Chicago, IL, USA).

Results

Participants

Descriptive participant characteristics for the COPD and control groups are displayed in Table 1. There were no between-group differences in age, height, body mass or body mass index (BMI). As expected, the COPD group had a significantly greater smoking history, increased airway obstruction, functional residual capacity and residual volume compared to controls (Table 1). COPD patients had significantly lower peak oxygen uptake but there was no evidence of arterial desaturation at rest or peak exercise in either group (Table 1).

Chemoreceptor reflex

The transient reduction in minute ventilation during hyperoxia and the slope of the ventilatory response to hypoxia were greater in COPD patients when compared to controls (Table 2). These data suggest that COPD patients have greater CC activity and sensitivity. There was no difference in the hyperoxic hypercapnic ventilatory responses between groups indicating that central chemoreceptor sensitivity was similar between COPD and controls (Table 2).

Respiratory responses to carotid chemoreceptor inhibition

See Table 3 for grouped cardiorespiratory data. Minute ventilation was similar between groups during saline infusion while breathing room air (Table 3). CC inhibition with hyperoxia and low-dose dopamine significantly reduced minute ventilation in COPD but had no effect in controls. When given both dopamine and hyperoxia, there was no further reduction in minute ventilation, suggesting that the interventions were not additive and the dopamine was likely a stronger CC inhibitor than inhaled O_2 .

Cardiovascular responses to carotid chemoreceptor inhibition

See Table 3, and Figs 1 and 2 for grouped cardiovascular data. Both central (cfPWV) and peripheral (crPWV) pulse-wave velocity were elevated in COPD during saline infusion while breathing room air when compared to controls (Fig. 1). Carotid–radial PWV was reduced with breathing 2 min of 100% O_2 in COPD but not in controls. Dopamine reduced both cfPWV and crPWV in COPD but not in controls (Fig. 1). MAP and pulse pressure were

Table 2. Central and carotid chemoreceptor reflex responses

	Control	COPD
Transient hyperoxia		
Baseline \dot{V}_E (L min ⁻¹)	7.88 ± 0.51	8.41 ± 0.83
Nadir (15 s) \dot{V}_E (L min ⁻¹)	7.76 ± 0.61	6.16 ± 0.61
Nadir (15 s) change in \dot{V}_E from baseline (L min ⁻¹)	0.12 ± 0.46	2.25 ± 0.59*
Hyperoxic hypercapnic ventilatory response		
$\Delta\dot{V}_E/\Delta P_{ETCO_2}$ slope (L min ⁻¹ mmHg ⁻¹)	1.22 ± 0.27	0.93 ± 0.18
Hypoxic ventilatory response		
$\Delta\dot{V}_E/\Delta S_{pO_2}$ slope (L min ⁻¹ % ⁻¹)	0.04 ± 0.03	0.23 ± 0.08*

Data are presented as means ± SEM. P_{ETCO_2} : partial pressure of carbon dioxide; S_{pO_2} : oxygen saturation measured by pulse oximeter; \dot{V}_E : minute ventilation. * $P < 0.05$ between groups

Table 3. Respiratory and cardiovascular data with either saline or dopamine while breathing room air or 2 min of hyperoxia (100% O₂) in controls and COPD

	Group	Saline normoxia	Saline hyperoxia	Dopamine normoxia	Dopamine hyperoxia
\dot{V}_E (L min ⁻¹)	Control	7.3 ± 0.4	7.9 ± 0.4	8.0 ± 0.7	8.2 ± 0.8
	COPD	8.5 ± 0.8	7.7 ± 0.7*	7.0 ± 0.6 [†]	7.3 ± 0.5 [†]
P_{ETCO_2} (mmHg)	Control	37.1 ± 1.3	34.8 ± 1.3	37.9 ± 1.6	34.9 ± 1.5
	COPD	34.0 ± 2.2	32.0 ± 2.1	36.2 ± 1.4	33.9 ± 1.7
P_{ETO_2} (mmHg)	Control	103.5 ± 1.9	576.8 ± 11.2 [#]	103.5 ± 2.1	562.6 ± 18.6 [#]
	COPD	110.5 ± 3.2	553.3 ± 19.2*	105.5 ± 2.2	567.7 ± 21.4*
S_{pO_2} (%)	Control	95.5 ± 0.7	98.7 ± 0.5 [#]	95.5 ± 0.6	99.0 ± 0.3 [#]
	COPD	94.3 ± 0.5	97.2 ± 0.7*	93.4 ± 0.6	97.6 ± 0.6*
\dot{Q} (L min ⁻¹)	Control	4.8 ± 0.3	5.0 ± 0.4	5.0 ± 0.4	5.1 ± 0.5
	COPD	4.5 ± 0.3	4.7 ± 0.3	4.6 ± 0.3	4.9 ± 0.3
HR (beats min ⁻¹)	Control	62.3 ± 2.4	62.1 ± 2.4	67.3 ± 2.8	65.1 ± 2.7
	COPD	62.2 ± 2.7	58.9 ± 2.7	64.8 ± 2.5	64.0 ± 2.7
SV (mL beat ⁻¹)	Control	78.8 ± 6.7	80.9 ± 6.4	75.1 ± 6.1	79.3 ± 6.5
	COPD	73.5 ± 5.0	80.6 ± 5.9	72.6 ± 6.3	83.3 ± 8.8
MAP (mmHg)	Control	91.0 ± 2.3	93.7 ± 2.5	91.9 ± 2.8	92.4 ± 2.9
	COPD	93.2 ± 2.1	92.7 ± 2.2	87.0 ± 2.9 [†]	85.7 ± 3.3 [†]
PP (mmHg)	Control	45.6 ± 3.3	45.5 ± 3.0	47.8 ± 3.3	47.5 ± 2.4
	COPD	48.9 ± 3.5	46.5 ± 2.7	42.4 ± 2.4 [†]	43.6 ± 3.0 [†]
\dot{Q}/MAP (mL min ⁻¹ mmHg ⁻¹)	Control	53.9 ± 4.7	53.7 ± 4.9	54.9 ± 4.6	56.0 ± 5.2
	COPD	47.7 ± 3.1	49.5 ± 3.0	53.8 ± 3.8*	57.6 ± 4.1*
Brachial artery diameter (mm)	Control	3.79 ± 0.18	3.78 ± 0.19	3.85 ± 0.17	3.83 ± 0.16
	COPD	3.97 ± 0.19	4.03 ± 0.18	4.08 ± 0.17 [†]	4.10 ± 0.19 [†]

Data are presented as means ± SEM. HR: heart rate; MAP: mean brachial arterial pressure; P_{ETCO_2} : partial pressure of carbon dioxide; P_{ETO_2} : partial pressure of oxygen; PP: brachial artery pulse pressure; \dot{Q} : cardiac output; \dot{Q}/MAP : vascular conductance; S_{pO_2} : oxygen saturation measured by pulse oximeter; SV: stroke volume; \dot{V}_E : minute ventilation. [#] $P < 0.05$ vs. control saline normoxia, * $P < 0.05$ vs. COPD saline normoxia, [†] $P < 0.05$ vs. control within condition.

significantly reduced with dopamine in COPD but not with hyperoxia (Table 3). No change in mean arterial pressure was observed in controls between conditions. Cardiac output, stroke volume, heart rate and brachial artery blood flow were unaffected by either hyperoxia or dopamine in both groups (Table 3, Fig. 2). Both brachial and total vascular conductance were significantly increased with dopamine in COPD but unchanged in controls, while conductance was unaffected with hyperoxia in either group (Table 3, Fig. 2). Brachial artery

diameter was similar between groups with saline (Table 3). Dopamine increased brachial artery diameter in COPD, while no change was observed in controls (Table 3).

In the subset of patients with MSNA recordings, there were no differences in resting burst frequency (42 ± 4 vs. 43 ± 8 bursts min⁻¹, $P = 0.61$) or burst incidence (64 ± 9 vs. 65 ± 13 bursts (100 heart beats)⁻¹, $P = 0.79$) between COPD patients and controls, respectively. CC inhibition with hyperoxia significantly decreased MSNA in COPD but not controls (Fig. 3).

Discussion

Carotid chemoreceptor inhibition with low-dose dopamine reduced central and peripheral arterial stiffness, and increased vascular conductance, in COPD patients, while no change was observed in risk-matched controls. Similarly, carotid chemoreceptor inhibition with inspired hyperoxia reduced both peripheral arterial stiffness and MSNA in COPD, while no effect was observed in controls. These data demonstrate the importance of the CC in contributing to the elevated arterial stiffness in COPD. There are multiple studies documenting elevated

central PWV in COPD patients; however, the potential mechanism(s) for the increased PWV in COPD remain unclear (McAllister *et al.* 2007; Sabit *et al.* 2007; Maclay *et al.* 2009). This is the first study to demonstrate that central PWV can be reduced acutely in COPD. This work, combined with previous work examining peripheral PWV responses to CC inhibition, demonstrates that the CC has an important influence on PWV and CV risk in mild–moderate COPD patients (Stickland *et al.* 2016).

The CC has been shown to be a significant sensor for both the control of breathing and CV function (Sun *et al.*

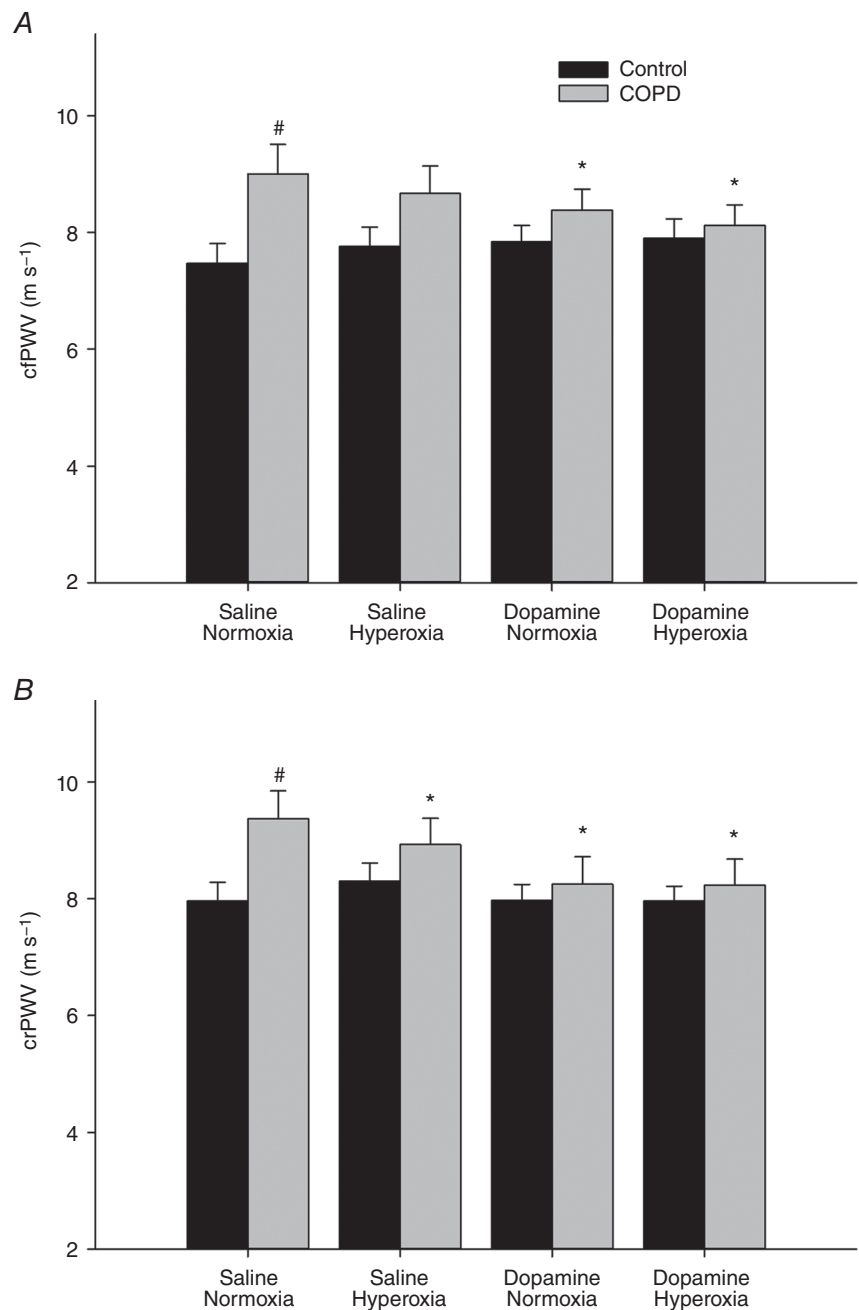


Figure 1. Carotid–femoral (cfPWV; **A**) and carotid–radial (crPWV; **B**) pulse-wave velocity with either saline or dopamine while breathing room air or 2 min of hyperoxia (100% O₂) in controls and COPD

Both cfPWV and crPWV were elevated in COPD compared to control, in the saline and normoxia condition. Dopamine reduced both cfPWV and crPWV in COPD, while hyperoxia reduced crPWV in COPD. [#]*P* < 0.05 vs. control saline normoxia, ^{*}*P* < 0.05 vs. COPD saline normoxia.

1999; Stickland *et al.* 2007). Importantly, it is unlikely that central chemoreception contributed to the observed reduction in PWV with dopamine, as dopamine does not cross the blood–brain barrier and would not stimulate the rostral ventrolateral medulla (Zlokovic, 2008). It is often assumed that low-dose dopamine causes vasodilatation through stimulation of dopamine-1 vascular receptors. However, low-dose dopamine does not cause vasodilatation in healthy controls (Stickland *et al.* 2011; Edgell *et al.* 2015), but vasodilatation is seen typically in conditions of high CC activity/sensitivity such as CHF (Stickland *et al.* 2007; Edgell *et al.* 2015) and at present COPD. It is acknowledged that the vasodilatation observed

with dopamine in COPD could be due to the direct peripheral vasodilatory actions of dopamine (via direct stimulation of dopamine-1 vascular receptors); however, it would be assumed that any direct peripheral vascular effects of dopamine would have also been observed in risk-matched controls. Results from the current study show that CC activity/sensitivity was elevated in COPD, and dopamine reduced ventilation and caused vasodilatation in these patients, while controls showed no response to dopamine. These results strongly suggest that the cardiovascular effects of low-dose dopamine in COPD are secondary to CC inhibition and not a central chemoreceptor or peripheral vascular effect.

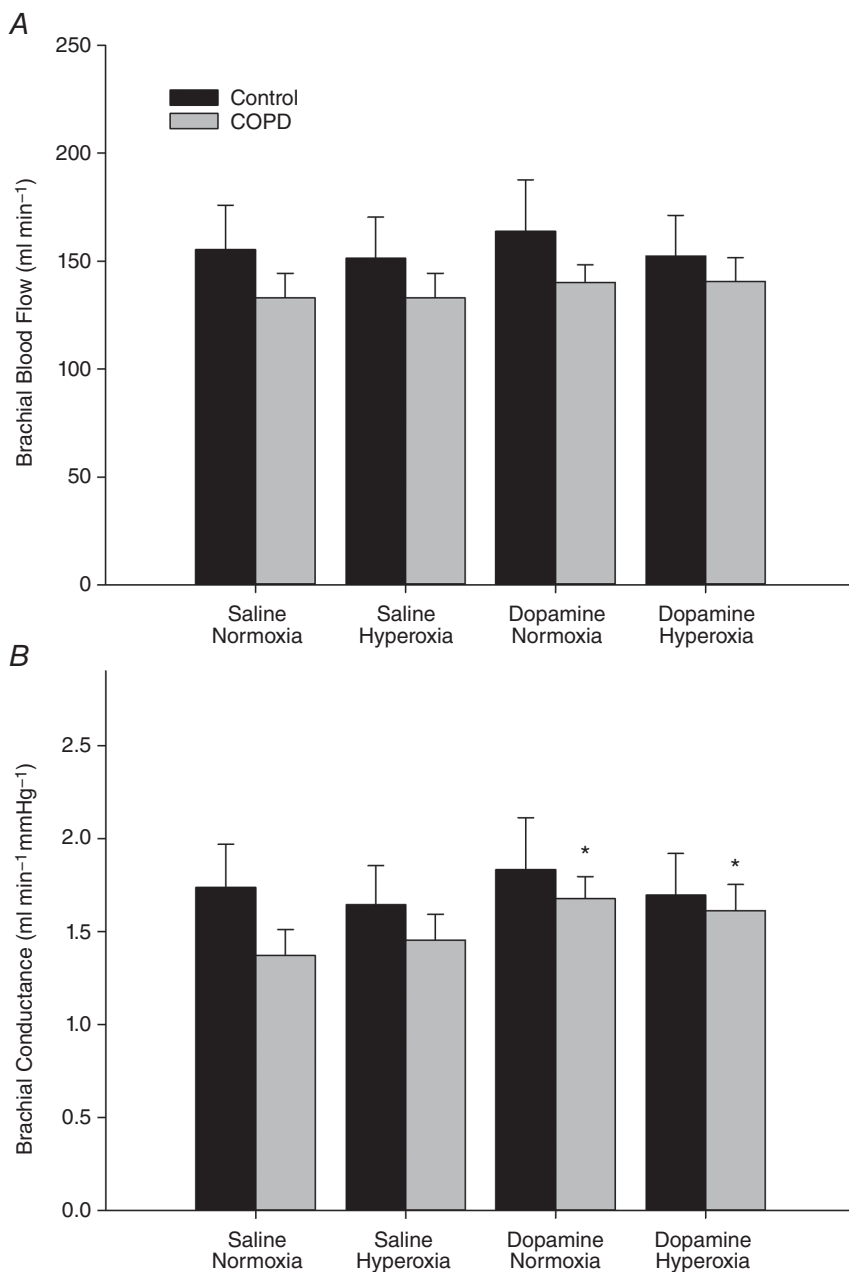


Figure 2. Brachial blood flow (A) and conductance (B) with either saline or dopamine while breathing room air or 2 min of hyperoxia (100% O₂) in controls and COPD

Dopamine increased conductance in COPD. *P < 0.05 vs. COPD saline normoxia.

Cardiovascular responses to carotid chemoreceptor inhibition

Arterial stiffness/PWV is determined by arterial wall material properties (e.g. elastin/collagen) and vessel diameter (O'Rourke *et al.* 2002). Neither short-term hyperoxia nor dopamine would acutely affect arterial wall properties. Rather, our data demonstrate vasodilatation (increased brachial artery diameter and vascular conductance) with CC inhibition, indicating that the

reduction in PWV with CC inhibition is likely secondary to increased vessel diameter. Changes in vessel diameter are highly influenced by MSNA (Laurent *et al.* 2006; Świerblewska *et al.* 2010) and increased MSNA has been linked with increased PWV, independent of age, body composition or blood pressure (Świerblewska *et al.* 2010). It is well documented that afferent carotid body discharge contributes to elevated SNA in health and CHF (Downing *et al.* 1962; Hardy *et al.* 1994; van de Borne *et al.* 1998), and inhibition of the CC reduces SNA and improves

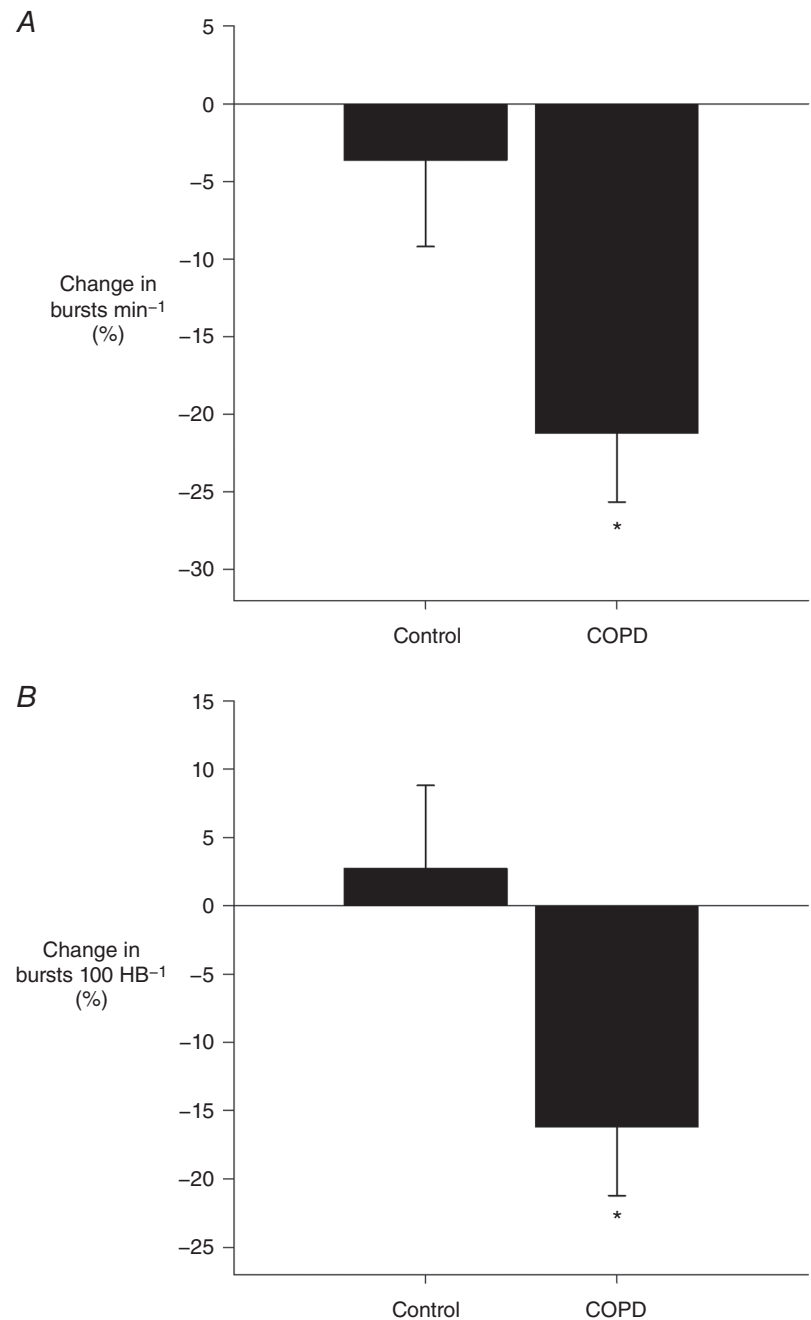


Figure 3. Change in muscle sympathetic nerve activity (MSNA) burst frequency (A) and burst incidence (B) from normoxia to hyperoxia in controls and COPD

MSNA was reduced with hyperoxia in COPD.

* $P < 0.05$ between groups.

CV function in CHF (Sun *et al.* 1999; Stickland *et al.* 2007; Edgell *et al.* 2015). MSNA is chronically elevated in COPD compared to healthy individuals (Raupach *et al.* 2008; Andreas *et al.* 2014), and in the current study, CC inhibition significantly reduced both peripheral and central arterial stiffness, and MSNA, in COPD patients. Based on well-established work linking the CC to MSNA, and MSNA to PWV, our data would indicate that the observed reduction in PWV with CC inhibition is likely the result of reduced MSNA.

Carotid–radial PWV reflects stiffness of the peripheral arteries, which are generally considered resistive vessels and thus under more smooth muscle sympathetic control compared to carotid–femoral PWV (i.e. central arterial stiffness). It is therefore not surprising that carotid–radial PWV had a larger reduction with dopamine than carotid–femoral PWV (0.9 ± 0.3 vs. 0.6 ± 0.3 m s⁻¹, respectively) in COPD patients. A meta-analysis concluded that a 1 m s⁻¹ increase in carotid–femoral PWV elevated CV mortality risk by 15% (Vlachopoulos *et al.* 2010). Based on these data, the 0.6 m s⁻¹ reduction in carotid–femoral PWV observed with CC inhibition in COPD patients in the current study would suggest an 8–9% reduction in risk of mortality with CC inhibition in COPD.

Chemoreflex

The reflex actions of both the central and peripheral (CC) chemoreceptors have been well characterized in other clinical conditions; however, there are limited studies in patients with COPD. Most of the previous work in COPD has focused on severe COPD patients who have complex physiological responses due to additional co-morbidities (e.g. heart failure), severe airway obstruction and chronic hypoxaemia (Bradley *et al.* 1979; Heindl *et al.* 2001; Ponikowski *et al.* 2001). Importantly, the patients in our study had mild–moderate COPD, were non-hypoxaemic and were compared to risk-matched controls without COPD. While the ventilatory response to hyperoxia (CC activity) and hypoxia (CC sensitivity) were significantly greater in COPD patients, there was no difference in the hyperoxic hypercapnic ventilatory response compared to risk-matched controls. These data suggest that while the peripheral (CC) chemoreceptors appear sensitized/activated in COPD, central chemoreception appears normal.

Limitations

Minute ventilation and peripheral arterial stiffness were reduced while breathing 100% O₂ in COPD, and these findings are similar to previous work in COPD (Stickland *et al.* 2016). Despite this evidence of CC inhibition, inhaled

hyperoxia did not have a similar effect to dopamine on central arterial stiffness and vascular conductance in COPD patients. It is important to note that arterial stiffness and blood flow measurements were taken in the second minute of the 2 min hyperoxic bout. It is possible that, consistent with previous work in healthy participants (Stickland *et al.* 2011), there was an acute vasodilatory response (<60 s) and a subsequent return to baseline, and that collecting data in the second minute resulted in our missing the transient vasodilatation. The lack of vasodilatation with hyperoxia may also be explained by auto-regulation as the increased oxygen content with hyperoxia would reduce blood flow to maintain O₂ delivery (Stickland *et al.* 2011). While the current results indicate that chronic suppression of CC activity may be an important target to reduce PWV and cardiovascular risk, care should be taken regarding the use of long-term hyperoxia, as this may have important deleterious secondary consequences (Bartels *et al.* 2004).

At moderate-to-high doses (i.e. 5–20 µg kg⁻¹ min⁻¹), dopamine can stimulate α- and β-receptors (Hoffman & Lefkowitz, 1990; Ciarka *et al.* 2007). β-receptor stimulation would be expected to increase heart rate; however, heart rate was unaffected in both groups with 2 µg kg⁻¹ min⁻¹ dopamine infusion, suggesting an absence of β-adrenergic stimulation. α-adrenergic stimulation would result in vasoconstriction, reducing conductance and increasing blood pressure; however, the opposite was observed in COPD patients. Therefore, the cardiovascular responses observed with dopamine in COPD patients are unlikely to be explained by α- or β-adrenergic stimulation.

Our groups were not matched for smoking history, and therefore we cannot separate the independent effect of smoking history vs. chronic airflow obstruction on chemoreception and vascular regulation.

While previous work has shown elevated resting MSNA in COPD (Raupach *et al.* 2008; Andreas *et al.* 2014), there was no between-group difference in baseline MSNA in our small subset of participants with MSNA data ($n = 7$ per group). Unfortunately, we were not able to obtain MSNA data in a larger sample of COPD patients because of technical difficulties in obtaining and maintaining the MSNA signal. Based on the previous research demonstrating neurohumoral activation in COPD (Hofford *et al.* 1990; Andreas *et al.* 2005, 2014; Raupach *et al.* 2008) and the variability in baseline MSNA observed in our controls, we were likely underpowered to detect a difference in baseline MSNA. Although no between-group difference in baseline MSNA was observed, our results combined with previous research examining carotid chemoreception and neurohumoral activation support the conclusion that the reduction in arterial stiffness was likely secondary to a reduction in MSNA.

Conclusions

CC inhibition reduced arterial stiffness and MSNA, and improved conductance in COPD. These data suggest that CC activity is increased at rest and contributes to elevated arterial stiffness in COPD. Our findings provide a potential mechanism for the increased PWV typically observed in COPD and demonstrate that the CC plays an important role in resting CV regulation in COPD. Interventions aimed at chronically reducing CC activity may be an effective strategy to reduce CV risk in COPD.

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Additional information

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Competing interests

None declared.

Author contributions

Conception or design of the work: D.B.P., V.T., M.B., M.K.S.; acquisition, or analysis or interpretation of data for the work: D.B.P., C.D.S., S.É.C., D.P.F., T.L.B., E.Y.L.W., M.K.S.; drafting the work or revising it critically for important intellectual content: D.B.P., C.D.S., S.É.C., D.P.F., T.L.B., E.Y.L.W., V.T., M.B., M.K.S. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed. This research was conducted through the Clinical Physiology Laboratory, Alberta Cardiovascular and Stroke Research Centre (ABACUS), Mazankowski Alberta Heart Institute.

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