ORIGINAL RESEARCH

Compromised Cerebrovascular Regulation and Cerebral Oxygenation in Pulmonary Arterial Hypertension

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***Background*—**Functional cerebrovascular regulatory mechanisms are important for maintaining constant cerebral blood flow and oxygen supply in heathy individuals and are altered in heart failure. We aim to examine whether pulmonary arterial hypertension (PAH) is associated with abnormal cerebrovascular regulation and lower cerebral oxygenation and their physiological and clinical consequences.

***Methods and Results*—**Resting mean flow velocity in the middle cerebral artery mean flow velocity in the middle cerebral artery ($MCAv_{mean}$); transcranial Doppler), cerebral pressure-flow relationship (assessed at rest and during squat-stand maneuvers; analyzed using transfer function analysis), cerebrovascular reactivity to CO_2, and central chemoreflex were assessed in 11 patients with PAH and 11 matched healthy controls. Both groups also completed an incremental ramp exercise protocol until exhaustion, during which $MCAv_{mean}$, mean arterial pressure, cardiac output (photoplethysmography), end-tidal partial pressure of CO_2, and cerebral oxygenation (near-infrared spectroscopy) were measured. Patients were characterized by a significant decrease in resting $MCAv_{mean}$ ($P<0.01$) and higher transfer function gain at rest and during squat-stand maneuvers (both $P<0.05$). Cerebrovascular reactivity to CO_2 was reduced ($P=0.03$), whereas central chemoreceptor sensitivity was increased in PAH ($P<0.01$), the latter correlating with increased resting ventilation ($R^2=0.47$; $P<0.05$) and the exercise ventilation/CO_2 production slope ($\dot{V}_E/\dot{V}CO_2$ slope; $R^2=0.62$; $P<0.05$) during exercise for patients. Exercise-induced increases in $MCAv_{mean}$ were limited in PAH ($P<0.05$). Reduced $MCAv_{mean}$ contributed to impaired cerebral oxygen delivery and oxygenation (both $P<0.05$), the latter correlating with exercise capacity in patients with PAH ($R^2=0.52$; $P=0.01$).

***Conclusions*—**These findings provide comprehensive evidence for physiologically and clinically relevant impairments in cerebral hemodynamic regulation and oxygenation in PAH. **(*J Am Heart Assoc*. 2017;6:e006126. DOI: 10.1161/JAHA.117.006126.)**

Key Words: central chemoreceptor sensitivity • cerebral ischemia • cerebral oxygenation • cerebrovascular reactivity to CO2 • exercise physiology

Pulmonary arterial hypertension (PAH) is characterized by distal pulmonary artery remodeling and a progressively failing right ventricle, resulting in poor exercise capacity.[1] Modern therapies have reduced short-term mortality and clinical worsening,[2,3] despite only minimal long-term improvement in survival and quality of life.[4,5] A better assessment of

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Accompanying Data S1, Table S1, and Figures S1 through S4 are available at http://jaha.ahajournals.org/content/6/10/e006126/DC1/embed/inline-supplementary-material-1.pdf

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Clinical Perspective

What Is New?

- Patients with pulmonary arterial hypertension (PAH) have an impaired cerebral pressure-flow relationship, indicating that changes in blood pressure are more passively transmitted to the brain.
- Patients with PAH also have lower cerebrovascular reactivity to CO_2 and increased central chemoreceptor sensitivity, the latter correlating with their exercise capacity.
- Patients exhibit marked impairments to cerebral blood flow and oxygenation during exercise, the latter tightly correlating with exercise capacity.

What Are the Clinical Implications?

- Impaired pressure-flow relationship likely contributes to presyncope symptoms and syncope in patients with PAH. Practically, therapies lowering blood pressure should be used cautiously in PAH, because brain perfusion is particularly sensitive to changes in blood pressure.
- Because cerebrovascular reactivity to CO_2 is an important determinant of cerebral blood flow, its dysregulation might contribute to decreasing cerebral blood flow in PAH.
- Increased central chemoreceptor sensitivity contributes to ventilatory abnormalities, resulting in increased resting ventilation and ventilatory inefficiency during exercise.
- Exercise-induced cerebral hypoxia might result in premature central fatigue and contribute to the multifaceted mechanisms of exercise intolerance in PAH.

the pathophysiological characteristics in PAH remains mandatory to address challenges in translating preclinical investigations into clinical trials and treatment.[6] As such, mechanisms involved in exercise intolerance remain incompletely understood. They include cardiopulmonary hemodynamic impairments and exercise-induced hypoxemia,[7] as well as impaired skeletal muscle function and morphological characteristics,[8,9] oxygenation[10] and angiogenesis.[10,11] However, other mechanisms may also contribute to limit exercise and daily activities in patients.

The regulation of cerebral blood flow (CBF) is complex. Its main determinants are blood pressure (BP) and the cerebral pressure-flow relationship, often termed cerebral autoregulation. Other determinants include cardiac output (CO), arterial blood gas, and autonomic nervous system and neurovascular coupling for meeting local cerebral metabolic demand.[12] Contemporary evidence demonstrates that CBF is reduced in patients with mild to severe heart failure (HF),[13,14] leading to cognitive dysfunction, brain anatomical changes, and exercise intolerance.[15,16] Potential mechanisms involved in CBF reduction remain unclear, but might be related to low CO,[17,18] lowered cerebrovascular reactivity to CO_2,[19,20] and an abnormal regulation of the cerebral pressure-flow relationship at rest[21,22] or during posture change.[18] Furthermore, in healthy individuals, intense exercise leads to cerebral deoxygenation, mild cerebral metabolic perturbation, and increased central fatigue in a similar manner as exercise in hypoxia, suggesting that cerebral oxygenation could affect exercise tolerance.[23] Similarly, reduced cerebral oxygenation per se has been demonstrated as a limiting factor for exercise tolerance in patients with HF[24] and type 2 diabetes mellitus,[25] among others.

Several mechanisms that might alter CBF regulation have been investigated in PAH over the years. As such, patients have been characterized by impaired baroreflex sensitivity,[26] increased sympathetic nerve traffic,[27] and hypocapnia.[28] Recently, reduced cerebral oxygenation and its impact on exercise capacity[29] and reduced cerebrovascular reactivity to CO_2[30] have been documented in PAH, using near-infrared spectroscopy; however, the associated integrative physiological mechanisms were not thoroughly investigated. Therefore, the aim of this study was to conduct a comprehensive assessment of the cerebrovascular function in patients with PAH to determine whether it is associated with abnormal cerebral vascular regulation and oxygenation and their physiological and clinical consequences. We hypothesized that patients with PAH would exhibit a disproportionate decrease in CBF as a result of vasoregulatory abnormalities. We also hypothesized that these abnormalities would lead to decreased cerebral oxygenation during exercise that would correlate with exercise tolerance.

Methods

Study Subjects

Eleven New York Heart Association functional class II to III patients with idiopathic and heritable PAH were recruited. PAH was defined as mean pulmonary arterial pressure $\geq$25 mm Hg at rest, with a pulmonary capillary wedge pressure $\leq$15 mm Hg, in agreement with the most recent guideline.[1] Only patients in stable condition during the past 4 months were eligible. Exclusion criteria were as follows: (1) a 6-minute walked distance of <300 m; (2) left ventricular ejection fraction <40%; (3) restrictive (lung fibrosis on computed tomography scan or total lung capacity <80% of predicted) or obstructive (forced expiratory volume in the first second of expiration/forced vital capacity <70%) lung disease; (4) body mass index >30 kg/m^2; and (5) metabolic diseases (eg, type 2 diabetes mellitus). Patients were individually matched for age, sex, body weight, and height with 11 healthy sedentary participants. All measurements were completed in a thermoneutral exercise physiology laboratory over 2 visits, separated by at least 48 hours (Figure S1). Participants were instructed to abstain from exercise, alcohol and caffeine consumption, and heavy meals in the 12 hours preceding

each experimental testing. The institutional ethics committee approved the research protocol (CÉR 20975), and all participants gave written informed consent.

Study Protocol and Measurements

CBF and central hemodynamics

On visit 1, CBF was estimated by monitoring $MCAv_{mean}$ (mean flow velocity in the middle cerebral artery) with transcranial Doppler ultrasonography (2.0-MHz probe; Doppler Box) using a standardized searching method.[31,32] After obtaining the optimal signal/noise ratio, the probe was secured with a custom-made headband and adhesive probe conductive ultrasonic gel (Tensive). Cerebrovascular resistance index (mean arterial pressure [MAP]/$MCAv_{mean}$), cerebrovascular conductance index ($MCAv_{mean}$/MAP), and Gosling pulsatility index ([systolic MCAv−diastolic MCAv]/$MCAv_{mean}$) were calculated. A 3-lead ECG was used for heart rate monitoring. BP was measured noninvasively by finger photoplethysmography (Nexfin; BMEYE). The cuff was positioned to the midphalanx of the middle finger of the right hand and calibrated at heart level. CO was determined by using a 3-element model of arterial input impedance (Modelflow method). Systemic pulsatility index was calculated as follows: (systolic AP−diastolic AP)/MAP. Photoplethysmographic and transcranial Doppler ultrasonographic data were simultaneously sampled at 1000 Hz via an analog-to-digital converter (Powerlab 16/30) and stored on an external disk for off-line computations (LabChart Pro version 7) (Data S1 provides further baseline analysis details).

Assessment and analysis of the cerebral pressure-flow relationship

Five minutes of spontaneous beat-to-beat BP and MCAv were recorded in seated rest position for all participants, followed by squat-stand maneuvers to enhance the linear interpretability of the pressure-flow metrics.[33,34] All participants started in a standing position, then squatted down until the back of their legs attained a 90° angle. This position was maintained for a specific period of time, after which they raised their legs. The time periods for squat-stand maneuvers were as follows: 10-second squat, 10-second stand and 5-second squat, and 5-second stand (performed in a random order).

The analysis approach has been developed and validated in previous studies.[33–35] Briefly, both BP and MCAv signals were time-aligned using −250 milliseconds, associated with the BP signal. Spontaneous (seated rest) and driven (squat-stand maneuvers) beat-to-beat BP and MCAv signals were spline interpolated and resampled at 4 Hz. Thereafter, transfer function analysis was performed on the basis of the Welch method. First, each high-pass filtered signal was divided into 5 segments, with 50% overlap between consecutive segments. Next, a Hanning window was applied to each data segment before fast Fourier transform analysis. The linear transfer function was derived as follows:

$$H_{PV}(f) = \frac{S_{PV}(f)}{S_{PP}(f)} \quad (1)$$

where S_{PV} is the cross-spectrum between BP and MCAv and S_{PP} is the autospectrum of BP. Spontaneous BP, MCAv spectral power, and the mean value of transfer function coherence, phase, and gain were calculated in the high frequency (0.20–0.40 Hz), low frequency (LF; 0.07–0.20 Hz), and very-low frequency (VLF; 0.02–0.07 Hz) ranges, as previously defined.[34] Previous studies have shown that transfer function gain may exhibit different dynamics, depending on whether the underlying time series are normalized.[36,37] Therefore, transfer function gain was assessed as normalized units (nGain; %/%) to account for the intersubject variability. For spontaneous data, the transfer function parameters were band averaged across the VLF, LF, and high-frequency ranges, but driven BP oscillations were sampled at the point estimate of the driven frequency (0.05 Hz corresponding to 10-second squat and 10-second stand, and 0.10 Hz corresponding to 5-second squat and 5-second stand). These point estimates were selected because they are in the VLF (0.02–0.07 Hz) and LF (0.07–0.20 Hz) ranges, where cerebral pressure-flow regulation is thought to be most operant.[33–35] Coherence quantifies to what extent changes in MCAv are linearly related with BP, whereas phase quantifies the temporal alignment between BP relative to MCAv.[33,34] The gain quantifies the effective amplitude dampening of BP fluctuations.[33,34] Only the transfer function analysis phase and gain values, where coherence >0.5, were included in analysis to ensure data robustness.[38] All data were analyzed with a custom-designed software in LabVIEW 2011. Respiratory gases and ventilation (Ultima $CardiO_2$ gas exchange analysis system) were monitored to ensure normal breathing in participants.

Cerebrovascular reactivity to CO_2 and central chemoreceptor sensitivity assessment

Both groups performed a modified rebreathing protocol.[39,40] Participants were seated comfortably and fitted with a mouthpiece connected to a pneumotach for respiratory gas analysis and a 2-way valve that allowed switching from room air to a 5-L rebreathing bag. The test consisted in 3 phases: (1) baseline (breathing room air); (2) hyperventilation to a target end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) between 20 and 25 mm Hg for 1 minute; and (3) dynamic rebreathing, during which participants were switched to the rebreathing bag with a gas mixture containing 7% CO_2 and 93% O_2 and breathed normally until the $P_{ET}CO_2$ was >60 mm Hg,

ventilation exceeded 100 L/min, or discomfort occurred. The cerebrovascular reactivity to CO_2 was determined using the linear regression of relative change (Δ) in $MCAv_{mean}$ against the $P_{ET}CO_2$. Both Δcerebrovascular conductance index-$P_{ET}CO_2$ and ΔMAP-$P_{ET}CO_2$ slopes were also assessed. A single line was fitted in the most linear part of the rebreathing phase to best exclude pressure-passive cerebral perfusion occurring after maximal vasodilatation. The central chemoreceptor sensitivity was determined by first plotting the minute ventilation ($\dot{V}_E$) and $P_{ET}CO_2$ to identify the $\dot{V}_E$ breakpoint (taken as the operating threshold of the central chemoreceptors) and then determined as the slope of a linear regression applied to the $\dot{V}_E$ against the $P_{ET}CO_2$ after the breakpoint (Data S1).

Incremental exercise test

On visit 2, a cardiopulmonary exercise test was performed on an electromagnetically braked cycle ergometer, according to current recommendations.[41] After a 5-minute rest and 1 minute of unloaded pedaling, participants exercised using an individualized incremental ramp protocol (5–25 W/min) until volitional exhaustion. Heart rate, BP, breath-by-breath analysis of respired gas, pulse oximetry, CO, and $MCAv_{mean}$ were continuously monitored, as previously described. Changes in cerebral oxygen delivery from baseline during exercise were estimated according to the following formula: $\Delta cDO_2 = \Delta MCAv_{mean} \times$ arterial oxygen content (estimated as $1.34 \times$ hemoglobin $\times \Delta$pulse oximetry). Exercise data from Nexfin and transcranial Doppler ultrasonography were resampled at 1 Hz and time aligned with breath-by-breath measurements. Data were then averaged over a minimum of 5 cardiac cycles and compared for relative workloads throughout the test.

Cerebral capillary oxygen saturation during exercise

Cerebral oxygenation was monitored by near-infrared spectroscopy (Oxiplex TS) in the prefrontal cortex capillary bed (Data S1). Relative changes from baseline in cerebral oxygenated, deoxygenated, and total hemoglobin concentration were measured.[10] Cerebral oxygenation saturation was evaluated using the cerebral oxygenated hemoglobin/cerebral total hemoglobin ratio, whereas change in cerebral deoxygenated hemoglobin was used as a surrogate of the local oxygen delivery and use matching.

Statistical Analysis

Data are reported as mean$\pm$SD, unless otherwise specified. An unpaired *t* test was used to compare both groups at baseline when data were normally distributed (D'Agostino-Pearson omnibus normality test and assessment of the SD). When not normally distributed, data were transformed using $\log_{10}$. Unpaired *t* tests were then applied to the transformed data. For exercise data, a 2-way repeated-measures ANOVA was used. After an interaction effect (exercise intensity$\times$groups), differences were located using paired (within-group) and independent (between-group) samples *t* tests, with Bonferroni correction. The Pearson correlation coefficient was used to evaluate the relationship between cerebral oxygenation saturation and change in cerebral deoxygenated hemoglobin and exercise capacity, defined as the percentage of predicted peak oxygen consumption ($\dot{V}O_2$peak % predicted). $P<0.05$ was considered statistically significant. Data were analyzed using GraphPad Prism version 6.0h for Mac.

Results

Patients With PAH Displayed Lower CBF at Rest

Baseline characteristics and exercise capacity variables in patients with PAH and controls are presented in Table. Resting MCAv (systolic, diastolic, and $MCAv_{mean}$; Figure 1A through 1C) was reduced in patients with PAH compared with controls, resulting in an increased Gosling pulsatility index (Figure 1D). Conversely, no differences in cerebrovascular conductance index and cerebrovascular resistance index were observed (Figure 1E and 1F).

PAH Is Characterized by Abnormal Cerebral Pressure-Flow Relationship

The spectral power of BP variability was decreased by 75% in patients with PAH during spontaneous oscillations in the LF range (0.07–0.20 Hz), whereas MCAv variability, coherence, and phase were similar between groups. However, nGain was increased by 21% in the LF range for patients (Figure 2). For driven oscillations, spectral power for BP and MCAv was lower in patients with PAH for both VLF and LF (Figure 3A through 3D). Transfer function analysis revealed similar coherence and higher phase shift for both frequencies; nGain was higher only in LF in patients with PAH (Figure 3E through 3G). Representative traces for 1 patient with PAH and 1 healthy control are shown in Figures S2 and S3. Ventilatory responses during driven oscillation assessment are presented in Table S1.

Lower Cerebrovascular CO_2 Reactivity and Increased Central Chemoreceptor Sensitivity to CO_2 in Patients With PAH

In patients, the cerebrovascular reactivity to CO_2 was significantly reduced, with a 58% and 78% decrease in the MCAv-$P_{ET}CO_2$ slope (Figure 4A) and cerebrovascular conductance index-$P_{ET}CO_2$ slope (Figure 4B), respectively.

Table. Baseline Characteristics and Exercise Capacity

Characteristics	PAH Group (n=11)	Control Group (n=11)
Demographics		
Sex, F/M ratio	8:3	8:3
Age, y	44 (12)	43 (15)
BMI, kg/m^2	25 (5)	25 (3)
PAH subtype		
iPAH/PAH-Her ratio	9:2	NA
WHO functional class		
II/III	8:3	NA
Resting pulmonary hemodynamic		
mPAP, mm Hg	49 (10)	NA
CI, L/min per m	3.2 (0.8)	NA
PVR, dyne/s per cm^5	547 (172)	NA
PAOP, mm Hg	10 (3)	NA
SvO_2, %	71 (5)	NA
Medical treatment with PAH-targeted agents		
Agents		
Prostacyclin analogue	2	NA
PDE-5i	9	NA
ERA	6	NA
Monotherapy	5	NA
Combination therapy	6	NA
Blood sampling		
Hemoglobin, g/L (PAH group, n=10; control group, n=11)	149 (15)	141 (15)
Hematocrit (PAH group, n=8; control group, n=6)	0.42 (0.03)	0.41 (0.03)
Rest hemodynamic and ventilation		
sAP, mm Hg	120 (21)	129 (11)
dAP, mm Hg	71 (12)	78 (7)
MAP, mm Hg	87 (14)	95 (7)
Systemic pulsatility index	0.561 (0.132)	0.537 (0.085)
HR, bpm	67 (14)	71 (9)
SpO_2, %	97 (2)	99 (1)*
$P_{ET}CO_2$, mm Hg	33 (4)	39 (5)†
V_E, L/min (PAH group, n=10; control group, n=11)	14 (2)	10 (2)‡
Exercise capacity		
6-Min walk test		
Distance, m	494 (93)	NA
% Predicted, %	85 (22)	NA
CPET		
Work rate, W	86 (34)	163 (54)‡
Peak $\dot{V}O_2$, mLO_2/min per kg	17.2 (4.0)	29.1 (5.7)§
Peak $\dot{V}O_2$ (% predicted)	67 (18)	110 (16)§
Anaerobic threshold, %	45 (11)	61 (12)†
$\dot{V}_E/\dot{V}CO_2$ slope	49 (15)	28 (5)§
End-exercise Borg dyspnea	7.9 (1.5)	7.8 (1.4)

Data are presented as mean (SD) unless otherwise indicated. Anaerobic threshold was determined using the V-slope method. BMI indicates body mass index; bpm, beats per minute; CI, cardiac index; CPET, cardiopulmonary exercise testing; dAP, diastolic arterial pressure; ERA, endothelin receptor antagonist; F, female; HR, heart rate; iPAH, idiopathic PAH; M, male; MAP, mean arterial pressure; mPAP, mean pulmonary arterial pressure; NA, not applicable; PAH, pulmonary arterial hypertension; PAH-Her, heritable PAH; PAOP, pulmonary arterial occlusion pressure; PDE-5i, phosphodiesterase type 5 inhibitor; $P_{ET}CO_2$, end-tidal CO_2 pressure; PVR, pulmonary vascular resistance; sAP, systolic arterial pressure; SpO_2, systemic oxygen saturation; SvO_2, systemic venous oxygen saturation; V_E, minute ventilation; V_E/VCO_2 slope, relationship between V_E and volume of expired CO_2; and WHO, World Health Organization.
*P<0.05.
†P<0.01.
‡P<0.001.
§P<0.0001.

Cerebrovascular reactivity to CO_2 was independent from MAP (Figure 4C). Hence, hyperventilation did not fully account for decreased CBF in patients with PAH because CBF was reduced for any given $P_{ET}CO_2$ value (Figure S4). Moreover, PAH was also associated with a 58% increase in central chemoreceptor sensitivity compared with controls (Figure 4D), which correlated with minute ventilation at rest (Figure 4E) and exercise $\dot{V}_E/\dot{V}CO_2$ slope in patients with PAH (Figure 4F).

Patients With PAH Displayed Lower $MCAv_{mean}$ and Cerebral Oxygenation During Exercise, Contributing to Exercise Intolerance

Patients with PAH displayed lower $MCAv_{mean}$ compared with controls throughout exercise (Figure 5A). Increases in CO and minute ventilation during exercise were comparable between groups, but MAP remained lower in patients compared with controls (Figure 5B through 5D). Patients with PAH also displayed a higher $\dot{V}_E/\dot{V}CO_2$ ratio and a lower $P_{ET}CO_2$ throughout exercise (Figure 5E and 5F).

Exploration of potential mechanisms driving CBF revealed that exercise-induced changes in $MCAv_{mean}$ in control subjects tightly correlated with changes in $P_{ET}CO_2$ (r=0.79; P=0.004), whereas it did not correlate with changes in MAP (r=0.36; P=0.35). Contrary to controls, in patients, exercise-induced changes in $MCAv_{mean}$ tightly correlated with changes

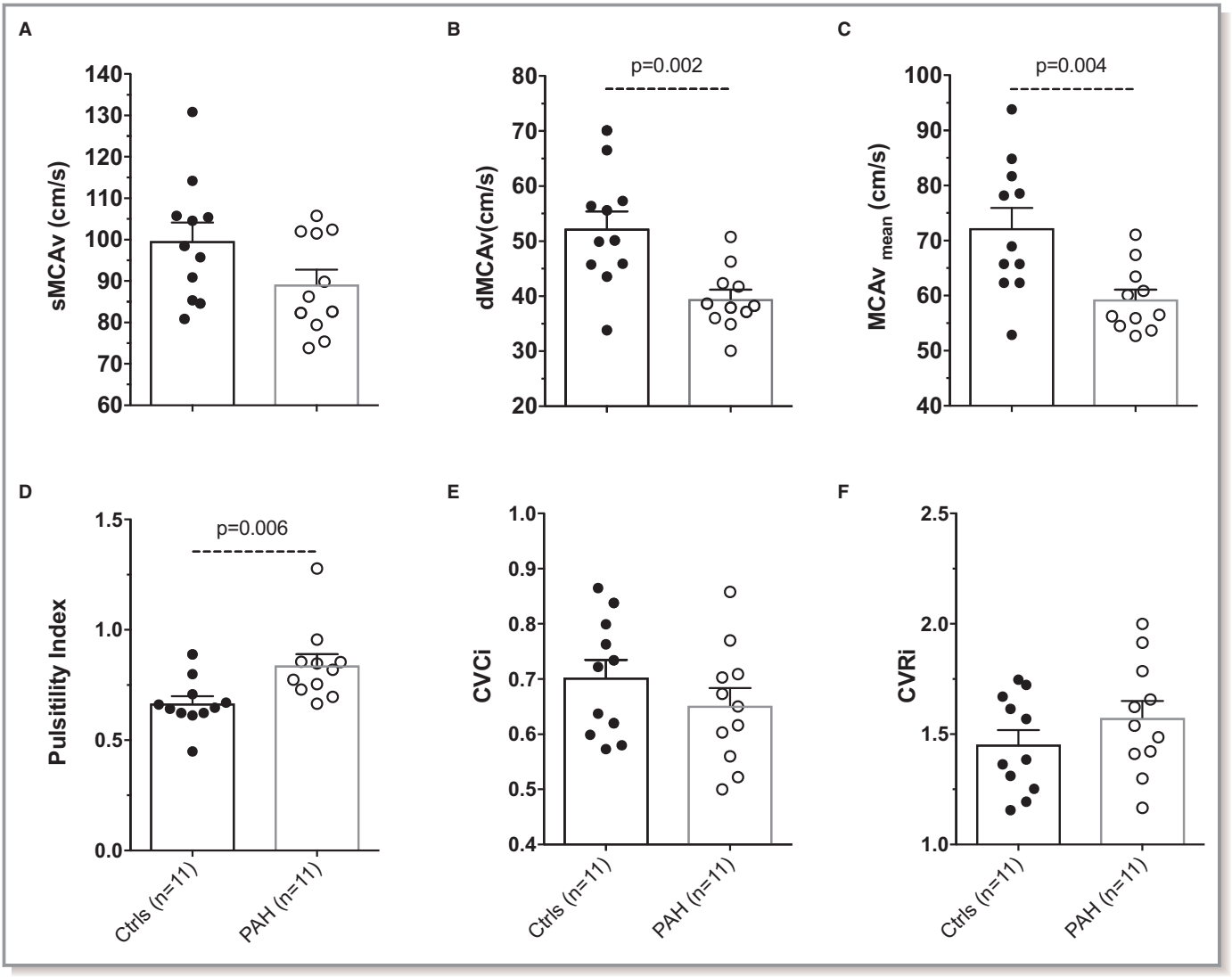

Figure 1. Resting cerebral blood flow (CBF) is reduced in patients with pulmonary arterial hypertension (PAH) compared with controls (Ctrls). Patients with PAH (open circles) exhibited lower resting CBF compared with Ctrls (closed circles), as assessed using systolic (P=0.08) (A), diastolic (P=0.002) (B), and mean flow velocity in the middle cerebral artery ($MCAv_{mean}$; P=0.004) (C). Consequently, patients with PAH exhibited increased Gosling pulsatility index (D), whereas cerebrovascular conductance index (CVCi; P=0.27) (E) and cerebrovascular resistance index (CVRi; P=0.25) (F) were similar. dMCAv indicates diastolic middle cerebral artery blood flow velocity; and sMCAv, systolic middle cerebral artery blood flow velocity.

in MAP (r=0.78; P=0.01), being consistent with the abnormal cerebral pressure-flow relationship previously observed. Conversely, exercise-induced changes in $MCAv_{mean}$ did not correlate with $P_{ET}CO_2$ (r=−0.42; P=0.19). Overall, these findings suggest that physiological mechanisms driving exercise-induced changes in $MCAv_{mean}$ differ between groups.

In addition to reduced $MCAv_{mean}$, patients with PAH had exercise-induced systemic oxygen desaturation (Figure 6A), contributing to decreased estimated cerebral oxygen delivery (Figure 6B). This finding resulted in markedly impaired cerebral oxygenation in PAH during exercise, beginning in the early stages of exercise (Figure 6C and 6D). End-exercise attenuated cerebral oxygenation saturation and end-exercise increased change in cerebral deoxygenated hemoglobin correlated with exercise capacity in patients with PAH only (Figure 6E and 6F).

Discussion

This study provides several new findings on cerebrovascular regulation and its impacts in PAH. We demonstrated that patients with PAH have a lower CBF at rest and during exercise, likely related to alterations in the cerebral pressure-flow relationship at rest and to lower systemic BP, throughout exercise. We also demonstrated that patients with PAH have a lower cerebrovascular reactivity to CO_2 and increased central

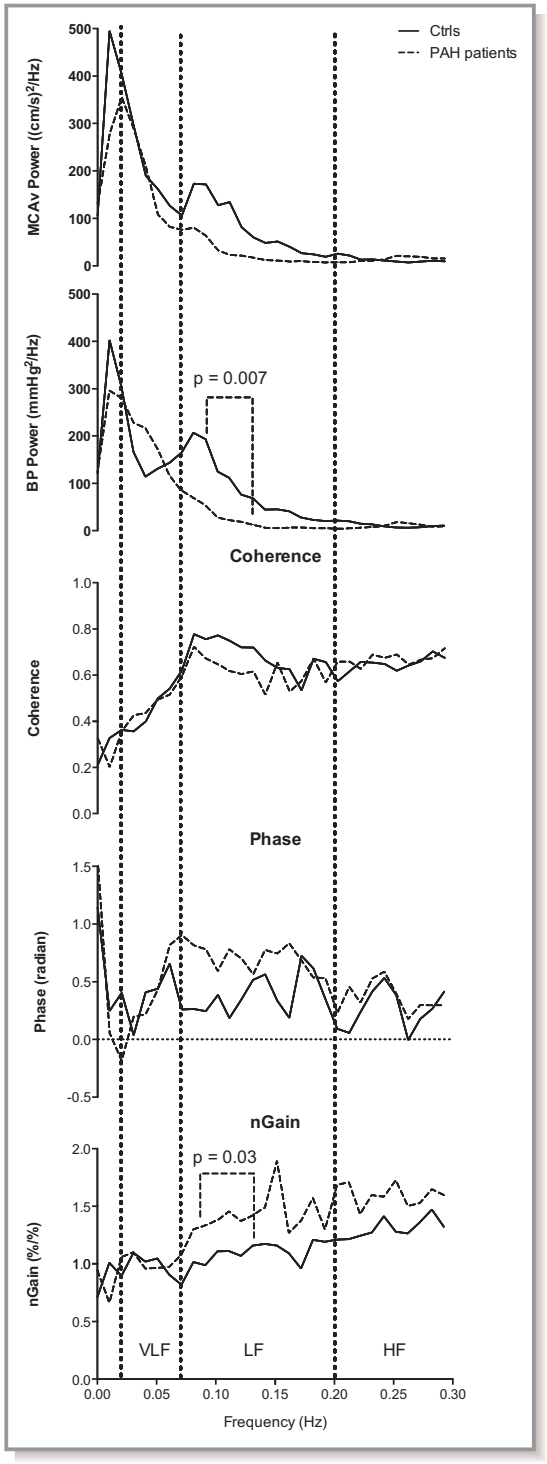

Figure 2. Spontaneous oscillation spectral power and transfer function analysis of resting cerebral blood flow and blood pressure (BP) for the entire spectrum from 0.00 to 0.30 Hz. Continuous (controls [Ctrls]; n=11) and dotted (patients with pulmonary arterial hypertension [PAH]; n=10) lines represent group-averaged middle cerebral artery blood flow velocity (MCAv) and BP spectral power, coherence, phase, and normalized gain (nGain). HF indicates high frequency (>0.20 Hz); LF, low frequency (0.07–0.20 Hz); and VLF, very low frequency (0.02–0.07 Hz).

chemoreceptor sensitivity that might further decrease brain perfusion. Ultimately, patients with PAH exhibit marked impairments in cerebral oxygenation, which tightly correlate with their exercise capacity.

Impaired Cerebral Pressure-Flow Relationship in PAH

Under both spontaneous and driven oscillations, patients had decreased BP and MCAv variability. Both metrics are important determinants of orthostatic tolerance. Indeed, maintained and even increased BP and MCAv variability have protected cerebral perfusion under experimental hypotension generated via low body negative pressure.[42] Nitric oxide release through increased cerebral shear stress has been suggested as one potential regulatory mechanism.[43] Interestingly, in addition to shear stress, exercise strongly promotes intravascular nitric oxide metabolite formation in healthy individuals, demonstrating that it accounts as an important mechanism for coupling oxygen delivery and metabolic demand.[44] Patients with PAH are well known for decreased nitric oxide synthesis, among other altered endothelium-dependent vasodilators.[45] Whether these alterations are also present in the cerebral vasculature of patients with PAH remains to be determined.

Under driven oscillations, patients with PAH have increased phase and nGain, altogether exhibiting alterations in the cerebral pressure-flow relationship. However, in healthy individuals, an impaired cerebral pressure-flow relationship is usually apparent through decreased phase and concomitant increased nGain.[33,34] Therefore, the present study revealed contrasting results under driven oscillations for the phase and nGain for patients with PAH. The phase was increased in both VLF and LF, whereas nGain was increased only in LF. One hypothesis might explain those results. The pronounced hypocapnic state and the increased demand on the respiratory system observed in patients may have contributed to the increased phase. Indeed, increased respiratory demand and acute hypocapnia both contribute to an increased phase in healthy young trained cyclists,[46] whereas hypocapnia occurring after the administration of endotoxin to healthy individuals was also associated with an increased phase.[47] The mechanisms altering the pressure-flow relationship are not well defined. However, one recent investigation in HF demonstrated that an increased cerebral microvascular tone, driven primarily through the tumor necrosis factor-$\propto$ that activates the sphingosine kinase 1/sphingosine-1-phosphate signaling pathway, accounts as one mechanism altering this relationship.[48] Interestingly, both elevated circulating proinflammatory cytokines and sphingosine kinase 1 and sphingosine-1-phosphate have been implicated in the characteristic proproliferative,

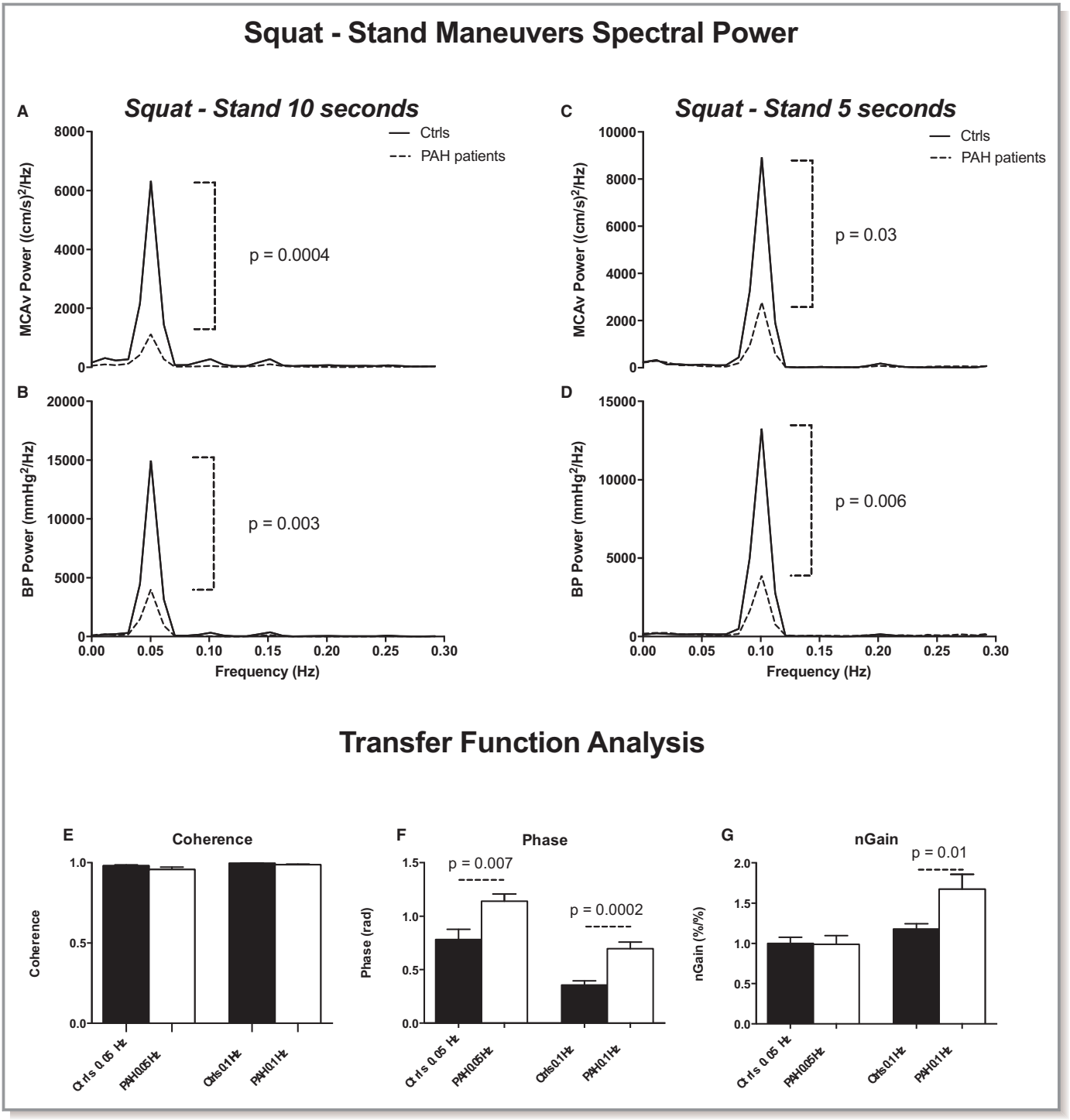

Figure 3. Driven oscillation spectral power and transfer function analysis of cerebral blood flow and blood pressure (BP). Group-averaged spectral power (A through D), coherence (E), phase (F), and normalized gain (nGain; G) for middle cerebral artery blood flow velocity (MCAv) and BP at 10-second squat-stand (0.05 Hz) and at 5-second squat-stand (0.10 Hz). Ctrls indicates controls; and PAH, pulmonary arterial hypertension.

antiapoptotic, and vasoconstrictive phenotype of PAH.[49–51] The potential implication of this pathway in the altered cerebrovascular regulation in PAH remains to be investigated.

Impaired Cerebrovascular Reactivity to CO_2 and Central Chemosensitivity in PAH

Cerebrovascular reactivity to CO_2 and cerebral pressure-flow relationship are important determinant of CBF regulation, but

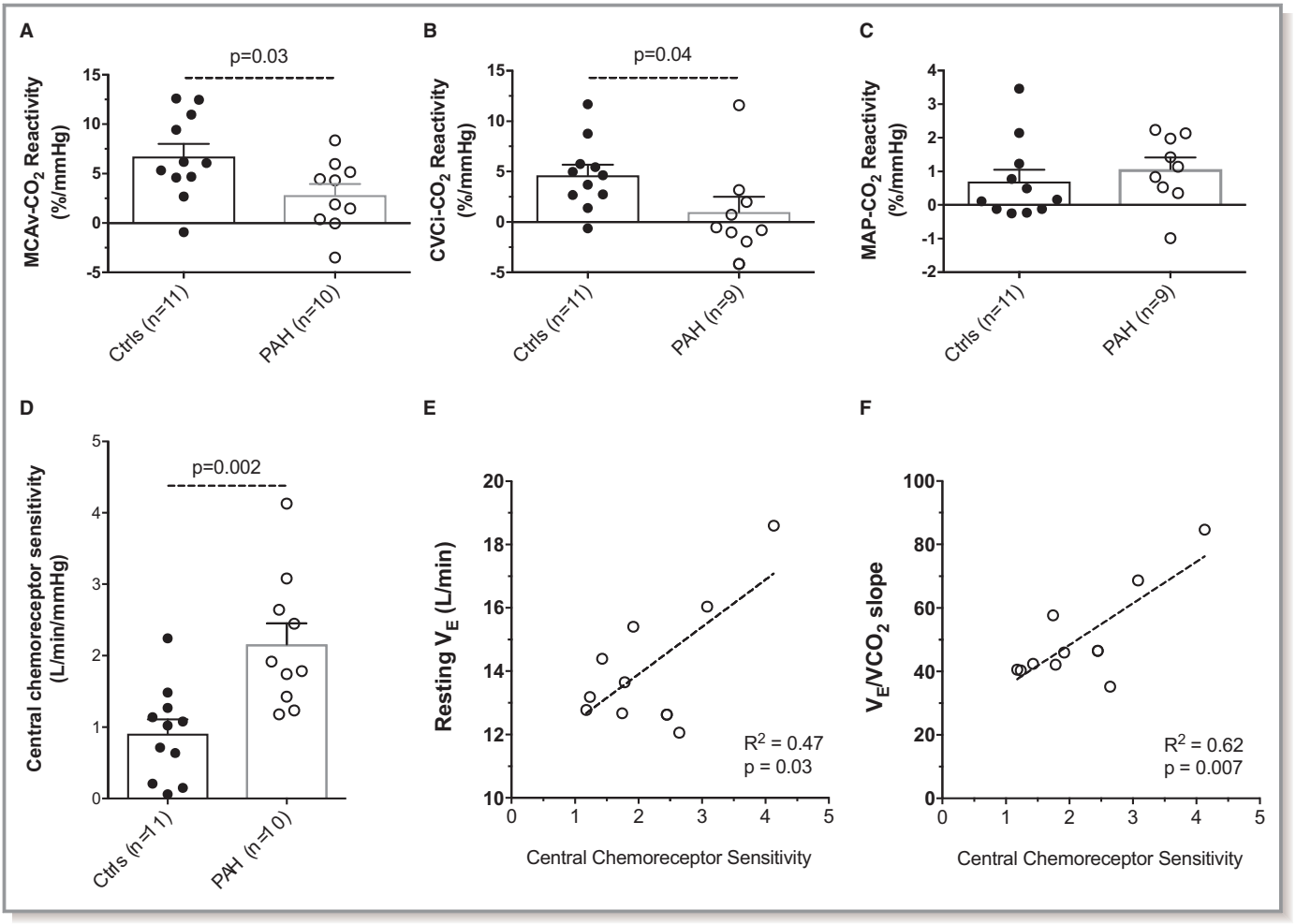

Figure 4. Cerebrovascular reactivity to CO_2 is decreased, whereas central chemoreceptor sensitivity is increased in patients with pulmonary arterial hypertension (PAH). Patients with PAH (open circles) exhibited a lower cerebrovascular reactivity to CO_2 (A) and a lower cerebrovascular conductance index (CVCi)–CO_2 reactivity (B) compared with controls (Ctrls; closed circles). No differences were observed between groups on the mean arterial pressure (MAP)–CO_2 reactivity (C). Patients had increased central chemoreceptor sensitivity compared with Ctrls (D). Among patients with PAH, chemoreceptor sensitivity correlated with minute ventilation at rest (E) and with $\dot{V}_E/\dot{V}CO_2$ slope during exercise (F). $MCAv_{mean}$ indicates middle cerebral artery mean blood flow velocity; $\dot{V}_E$, minute ventilation; and $\dot{V}_E/\dot{V}CO_2$ slope, ventilatory equivalent for CO_2 slope.

controlled through different mechanisms.[36,52] CO_2 modulates CBF via a direct effect of extracellular pH on the smooth muscle cells of small pial arterioles, in addition to other neural, humoral, and physical factors.[12] Hence, an increase in CO_2 leads to acidosis that decreases vascular smooth muscle cells' tone through activation of potassium (K^+) channels,[52] including the ATP-sensitive K^+ channels, voltage-gated K^+ channels, and 2-pore domain weakly inward rectifying (TWIK)-related acid sensitive (TASK-1) K^+ channelfamily member,[53] resulting in increased CBF. Interestingly, several voltage-gated K^+ channel expressions, including TASK-1 expression, are decreased in the lungs of patients with PAH.[54,55] Whether those defects are limited to the lungs remains elusive.

Recently, Vicenzi et al indirectly measured increased central chemosensitivity in PAH, based on the method of Naeije and van de Borne,[56] by plotting the exercise ventilatory equivalent for CO_2 ($\dot{V}_E/\dot{V}CO_2$) against $P_{ET}CO_2$, suggesting it might play a role in the wasted ventilation.[57] The present study directly assessed central chemoreceptor sensitivity and demonstrates its increase in PAH as well. Dyspnea is traditionally associated with ventilatory abnormalities, characterized by higher ventilatory demand, dynamic hyperinflation, and a rapid, shallow breath frequency, especially in patients with severe PAH.[58,59] When compared with HF, patients with PAH have similar exercise intolerance, but are more dyspneic in relation to reduced ventilatory efficiency.[60] Therefore, the present data add to the current literature on dyspnea in PAH, because increased central chemoreceptor sensitivity contributes to increased resting ventilation and increased ventilatory inefficiency during exercise. Hence, as suggested by Vicenzi et al, increased chemosensitivity is an important mechanism in wasted ventilation for patients.[57]

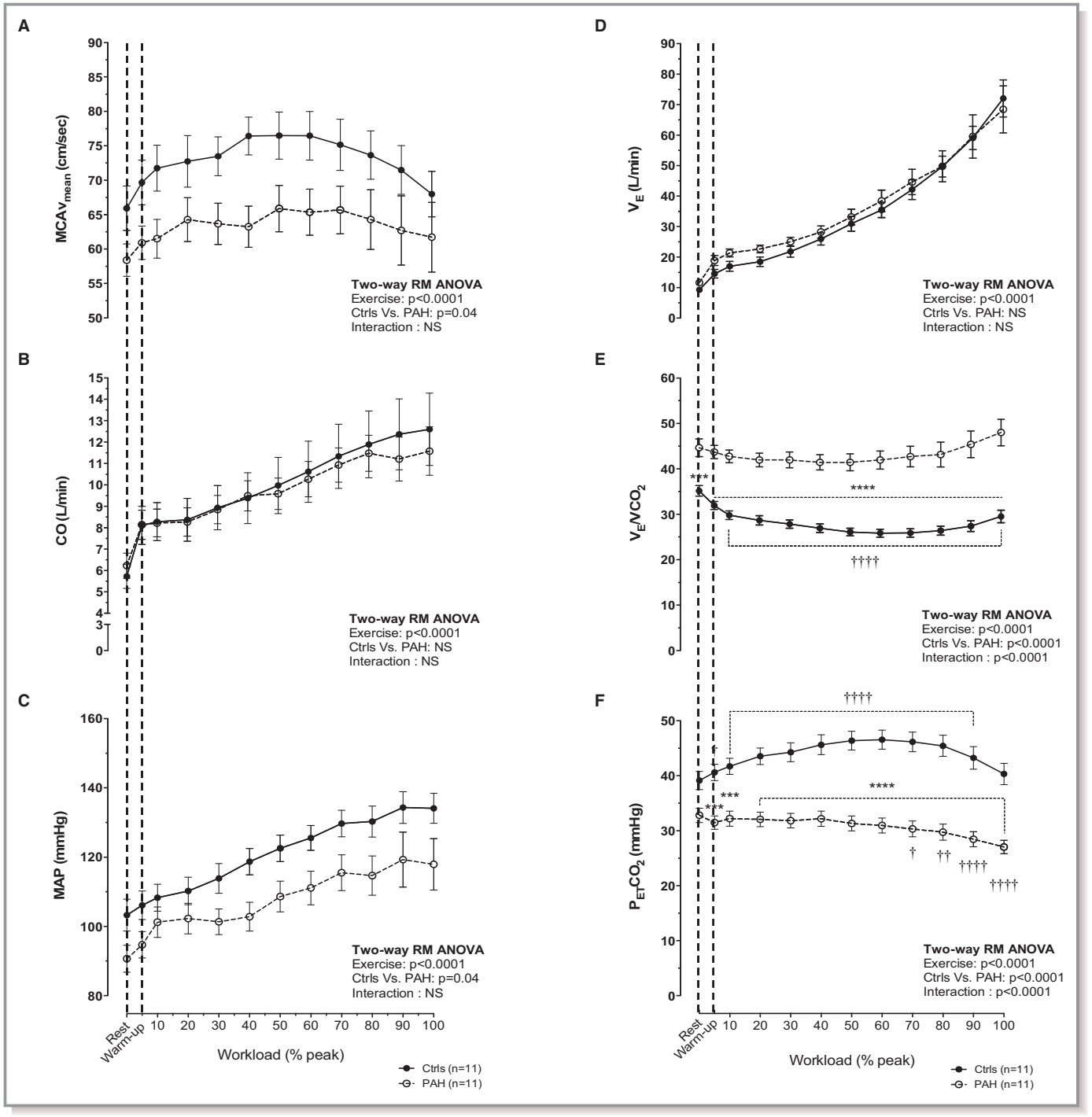

Figure 5. Cerebral blood flow and central hemodynamic and ventilatory response in patients with pulmonary arterial hypertension (PAH) and controls (Ctrls) during cardiopulmonary exercise testing. Patients with PAH (open circles) exhibited limited increases in middle cerebral artery mean blood flow velocity (MCAv$_{mean}$) compared with Ctrls (closed circles) (A, P=0.04), despite similar increases in cardiac output (CO; P=0.96; B). Patients with PAH also had a smaller increase in mean arterial pressure (MAP; P=0.04) compared with Ctrls (C). Although patients with PAH and Ctrls had comparable minute ventilation (V_E) at their maximal workload (D), patients with PAH had a higher ventilatory equivalent for CO_2 ($\dot{V}_E/\dot{V}CO_2$) (P<0.0001; E) and a persistently lower end-tidal pressure in CO_2 ($P_{ET}CO_2$) compared with Ctrls (P<0.0001; F). The x axis represents the different stages of exercise (rest, unloaded pedaling, and 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% of maximal workload). The unloaded pedaling is the average of the last 30 seconds before workload onset. Resting MCAv$_{mean}$, resting hemodynamic and ventilatory responses are calculated as the average of the last stable minute before beginning unloaded pedaling. Each MCAv$_{mean}$, hemodynamic, and ventilatory response data point represents a 10-second average for its relative workload. NS indicates nonsignificant; RM, repeated measures. Significantly different from rest: $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, $^{\dagger\dagger\dagger\dagger}P$<0.0001. Patients with PAH vs Ctrls: ***P<0.001, ****P<0.0001.

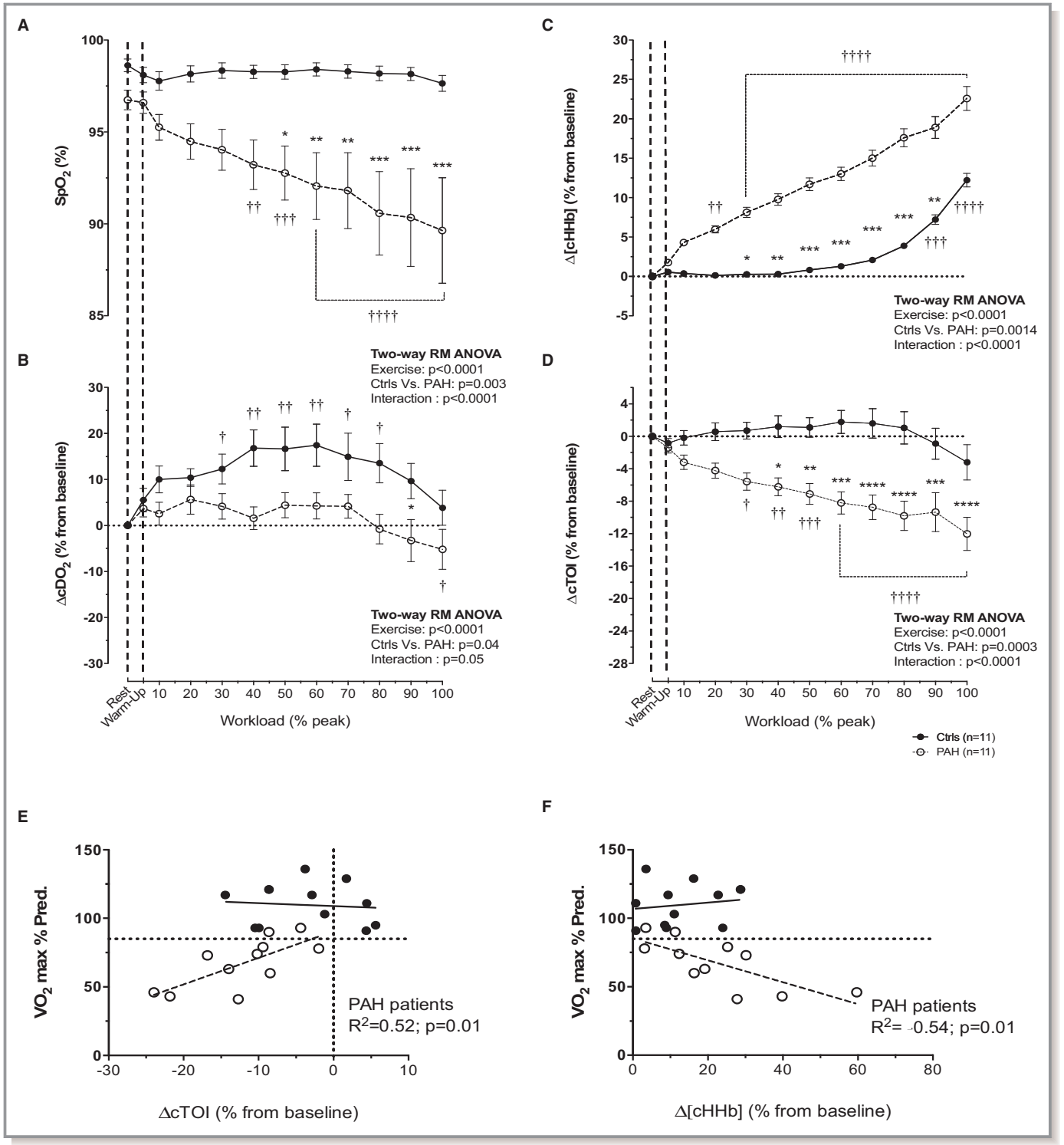

Figure 6. Cerebral oxygen capillary saturation is impaired and correlates with exercise tolerance in pulmonary arterial hypertension (PAH). Patients with PAH (open circles) exhibited a rapid and constant decrease in systemic oxygen saturation (SpO_2) at exercise (A), contributing to a significantly lower estimated cerebral oxygen delivery (cDO_2), expressed as changes from baseline (B). Patients with PAH exhibited a sustained decrease in cerebral tissue oxygenation index (ΔcTOI), while exhibiting a sustained increased in cerebral deoxyhemoglobin (Δ[cHHb]) compared with controls (Ctrls; closed circles; C and D). Lower ΔcTOI and increased Δ[cHHb] correlated with maximal exercise capacity in patients with PAH (E and F). The *x* axis represents the different stages of exercise (rest, unloaded pedaling, and 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% of maximal workload). The unloaded pedaling is the average of the last 30 seconds before workload onset. RM indicates repeated measures; and VO_2max % pred., predicted value of maximal oxygen consumption. Significantly different from rest: $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$, $^{\dagger\dagger\dagger\dagger}P<0.0001$. Patients with PAH vs Ctrls: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, **** $P<0.0001$.

Indeed, the cerebrovascular reactivity to CO_2 ultimately serves to keep central pH relatively constant, washing out brain stem CO_2 through vasodilation. In this context, a blunted cerebrovascular reactivity to CO_2 might protect the patients' brain from further decrease in perfusion, but might also add to the stimulation of the central chemoreflex by preventing adequate CO_2 washout from the brain stem.

Cerebral Oxygenation

Although brain oxygen demand increases with exercise, brain oxygenation is only secured by enhancing perfusion or oxygen extraction. In the present study, cerebral capillary oxygen saturation was assessed noninvasively using near-infrared spectroscopy, which has adequately tracked changes in jugular bulb saturation.[61] In PAH, cerebral oxygenation was markedly impaired during the early stages of exercise and persisted until exhaustion as a result of both exercise-induced systemic desaturation and reduced CBF. Impaired cerebral oxygenation also strongly correlated with exercise capacity in PAH, as did skeletal muscle oxygenation.[10] Therefore, this finding supports that cerebral oxygenation is critical for exercise in patients. However, whether cerebral oxygenation specifically influences exercise tolerance in PAH per se, or simply reflects global impairment in oxygen transport and use, remains to be determined.

Clinical Consequences

Impaired cerebral pressure-flow relationship indicates that changes in BP are more passively transmitted to the brain in PAH and are likely to explain why patients are less tolerant to presyncope symptoms and more prone to syncope after Valsalva-induced decreases in BP.[62] Pharmacological treatment plans should take into consideration that brain perfusion is particularly sensitive to BP impairments in patients with PAH. Exercise-induced cerebral hypoxia might lead to a shift toward nonoxidative metabolization of lactate and glucose, in a similar manner as short-term altitude exposure in healthy individuals,[63] increasing central fatigue[64] and contributing to early exercise termination. Prevalent mental disturbance has been associated with HF, including multiple brain anatomical and cognitive changes.[15,65,66] Different investigators considered that impaired CBF might be one contributing factor.[66,67] Therefore, the impact of cerebral hypoxemia, potential brain anatomical dysfunction, and cognitive dysfunction could be present in PAH and needs further investigations.

Methodological Limitations

First, we acknowledge that the limited sample size and the multiple statistical analyses might have led to type I errors. However, our sample size was calculated a priori and was comparable to similar studies in PAH. More important, all physiological observations were consistent with impairments in cerebral hemodynamic regulation and oxygenation in PAH, reinforcing the external validity of our findings. Also, the skull is an obstacle to the penetration of ultrasound waves because the bone strongly attenuates their resolution, making measurement of vessel luminal diameter not possible with transcranial Doppler ultrasonography.[32] The validity of $MCAv_{mean}$ as a surrogate of CBF assumes a constant diameter of the insonated vessel.[31] This assumption has been valid with arterial blood gas changes.[68,69] However, it remains possible that diameter changes occur in similar condition.[70,71] In the present study, the lower $P_{ET}CO_2$ in PAH would tend to reduce the MCA diameter, overestimating CBF and thus minimizing differences between PAH and controls. Second, squat-stand maneuvers can be considered as aerobic exercise. Thus, these maneuvers may modulate the dynamic pressure-flow relationship of the cerebral circulation by increasing regional brain activation and, more systemically, resulting in autonomic and humoral changes that may modulate CBF, especially in patients with PAH. The impact of brain activation during the squat-stand maneuvers on both phase and nGain should be minimal because moderate- and high-intensity aerobic exercise does not alter cerebral pressure-flow relationship.[72] Transfer function analysis metrics rely on a linear mathematical approach to interpret the linear relationship between BP and CBF. However, recent studies suggest that other dynamic mechanisms, such as cerebral vessel compliance, might play a role in dampening BP fluctuations, the latter being nonlinear.[73] Therefore, these results cannot be interpreted as measures of cerebral autoregulation per se.

Likewise to consider, acute severe hypoxia may limit the extent of CO_2-mediated vasoconstriction through blunted cerebrovascular reactivity to CO_2 to preserve CBF and metabolism.[74] This could have minimized the differences in CBF observed between patients with PAH and controls. However, most of our patients had normal or near-normal systemic oxygenation at rest. Therefore, hypoxic effect on the response of CBF to CO_2 is likely negligible. Also, we acknowledge that hypocapnia and lactic acid accumulation at the early stage of exercise could have resulted in a leftward and a rightward shift of the hemoglobin dissociation curve, respectively, modulating O_2 affinity to hemoglobin and potentially adding to the burden of lower cerebral oxygen delivery and oxygenation. Finally, both iloprost and sildenafil only minimally influence short-term flow velocity in the posterior cerebral artery in PAH.[75] Nevertheless, a potential influence of the PAH therapies cannot be excluded.

Conclusion

The present study provides evidence that patients with PAH have functional cerebrovascular abnormalities, including an

impaired cerebral pressure-flow relationship and a blunted cerebrovascular reactivity to CO_2. Patients with PAH also exhibit enhanced chemoreceptor sensitivity in relation to excessive ventilation at rest and effort. During exercise, these functional abnormalities are also present and contribute, in addition to exercise-induced hypoxemia, to impair cerebral oxygenation, which correlates with exercise capacity in PAH.

Authors' Contribution

Malenfant, Brassard, and Provencher contributed to the original idea of the study. Malenfant supervised the patients' recruitment. Malenfant, Paquette, Le Blanc, Chouinard, and Nadeau were responsible for collecting data. Simard and Bonnet contributed to data collection and interpretation. Malenfant, Allan, Tzeng, and Brassard analyzed and interpreted the data. Malenfant, Brassard, Tzeng, and Provencher drafted the article. All authors provided approval of the final article.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Measurements

Cerebral capillary oxygen saturation. Cerebral oxygenation was monitored by near-infrared spectroscopy (NIRS) using a single-distance, continuous wave light, dual-channel Oxiplex TS (ISS, Champlain, IL, USA). This system exploits the difference in optical absorption spectra between the oxygenated and the deoxygenated state of the hemoglobin in small vessels $\leq$200 μm, providing an estimated capillary O_2 saturation. The NIRS fiber optode consisted of eight light-emitting diodes operating at wavelengths of 690 and 830 nm and one detector with a separated distance of approximately 4 cm, corresponding to a light penetration depth of 2 cm in the brain tissue. The signal was analyzed using the modified Beer-Lambert law. Before placements, the NIRS system was calibrated using calibration block of known density. Values reported for cerebral oxygenation account predominantly for hemoglobin oxygenation in the pre-frontal cortex. The NIRS probe was attached as high as possible on the left forehead to avoid the frontal sinuses, and was covered by a black bathing cap and the headband for protection from external light and to limit probe movements.

Data Analysis

Baseline analysis. Baseline cerebral and central hemodynamics were both calculated as the average of the last stable minute of the seated resting period for day 1 and before CPET. Nexfin has shown reliably to assess the dynamic changes in beat-to-beat BP and cardiac output [1,

[2]. The Nexfin use has also been validated against intra-aortic BP measurements for frequency-domain patterns for coherence, phase and gain response [3].

Absolute cerebrovascular reactivity to CO_2 Analysis. The cerebrovascular reactivity to CO_2 was also determined using absolute $MCAv_{mean}$ (cm/s). $MCAv_{mean}$ and $P_{ET}CO_2$ were pooled using a method for averaging the individual response curves, thereby minimizing intersubject variability while preserving intrinsic linearity [4]. Data from the last minute of the baseline phase, the last 30 sec of the hyperventilation phase and the rebreathing phase, one minute after switching to the rebreathing bag, were used. Linear regression was then used to obtain the slope of the pooled $MCAv_{mean}$ vs. $P_{ET}CO_2$ relationship. Data are presented in **Figure S4**.

Table S1. Ventilatory responses during rest and the squat-stand manoeuvres

	PAH	Ctrls	p-Value
Seated Rest	**n=10**	**n=11**	
V_E (L/min)	11 (3)	8 (2)	0.01
RR (Br/min)	17 (4)	12 (3)	<0.01
V_T (L)	0.67 (0.14)	0.66 (0.12)	0.91
End-tidal CO_2 (mmHg)	32 (5)	41 (5)	<0.01
CV End-tidal CO_2 (%)	3.2 (0.9)	3.2 (0.9)	0.99
Repeated squat-stand maneuvers (0.05 Hz)	**n=10**	**n=11**	
V_E (L/min)	16 (2)	12 (2)	<0.01
RR (Br/min)	21 (5)	17 (3)	0.02
V_T (L)	0.84 (0.37)	0.75 (0.10)	0.58
End-tidal CO_2 (mmHg)	32 (4)	42 (6)	<0.01
CV End-tidal CO_2 (%)	3.5 (0.9)	5.3 (1.5)	<0.01
Repeated squat-stand maneuvers (0.10 Hz)	**n=9**	**n=11**	
V_E (L/min)	17 (3)	13 (2)	<0.01
RR (Br/min)	22 (5)	17 (3)	<0.01
V_T (L)	0.84 (0.29)	0.79 (0.09)	0.60
End-tidal CO_2 (mmHg)	33 (4)	42 (6)	<0.01
CV End-tidal CO_2 (%)	3.8 (2.1)	5.6 (1.6)	0.04

Data are presented as mean (standard deviation). Abbreviations are V_E: minute ventilation; RR respiratory rate; V_T: tidal volume; CV: coefficient of variation.

Figure S1. Experimental protocol.

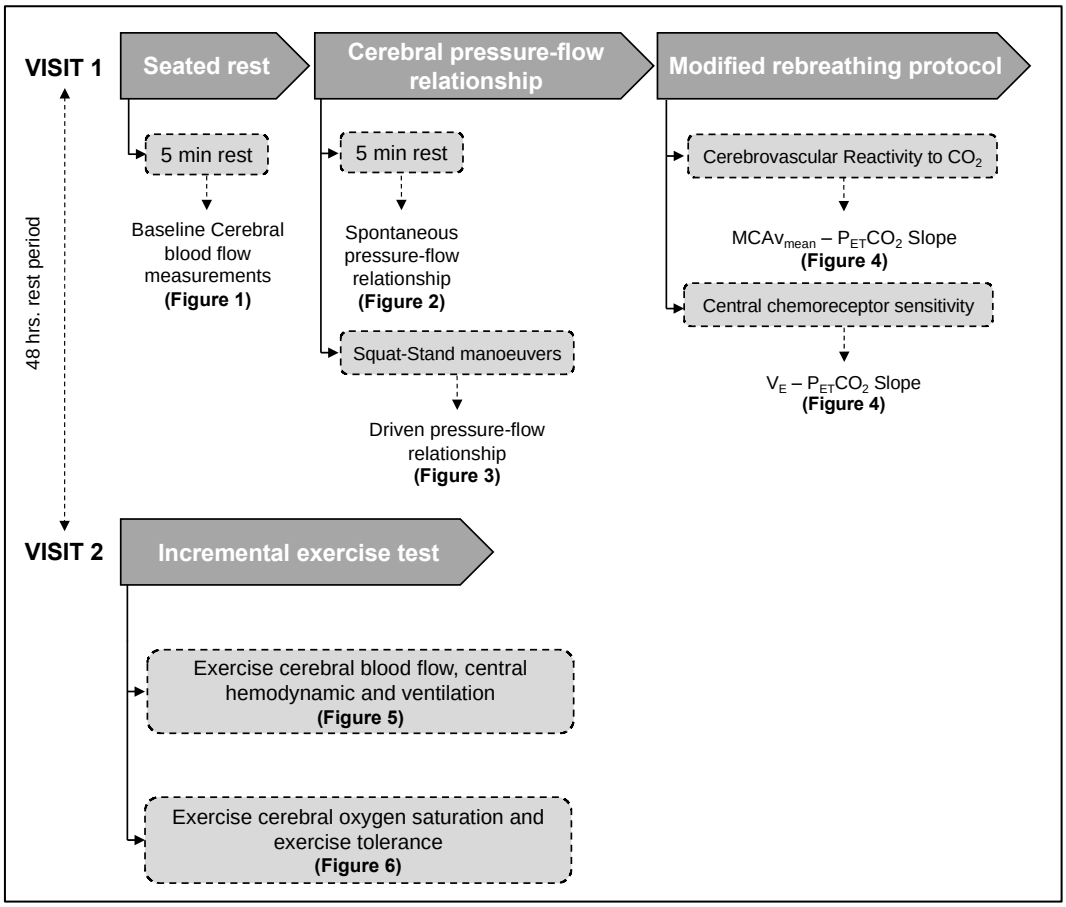
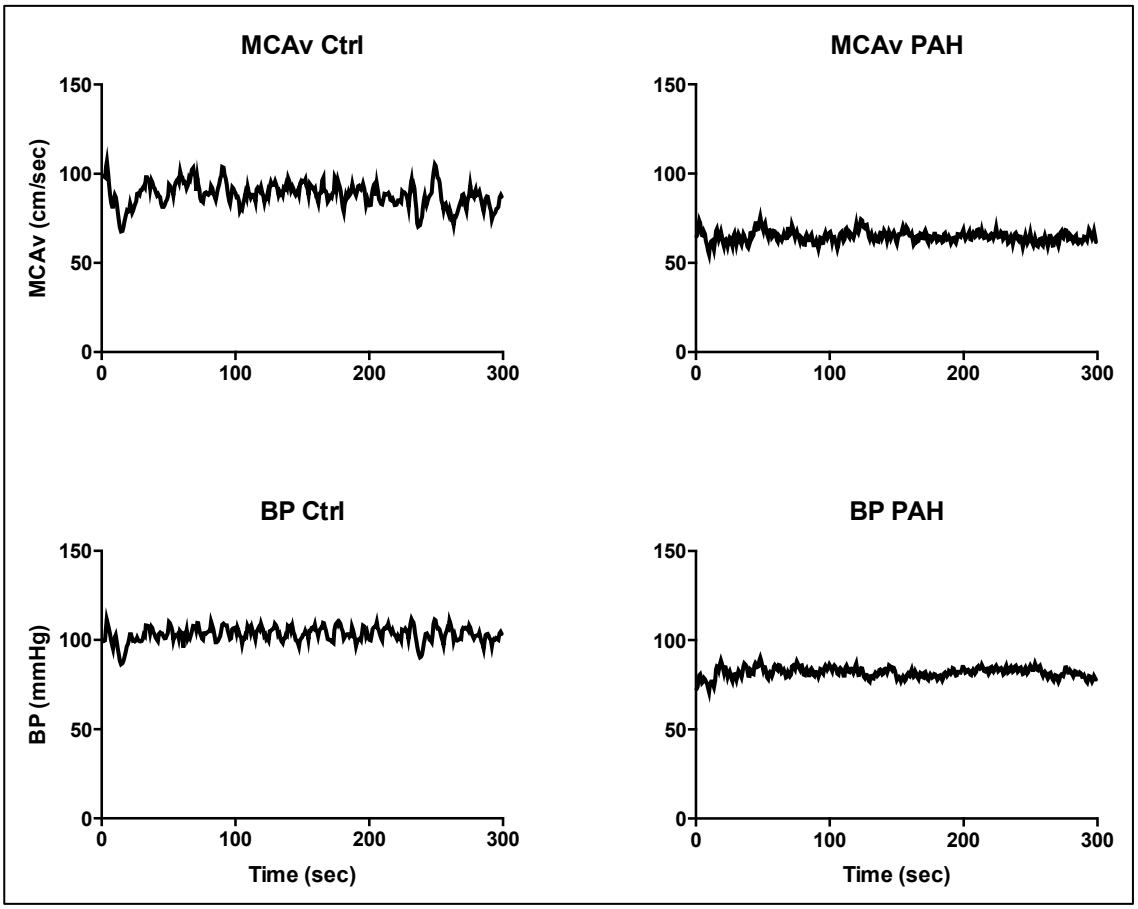
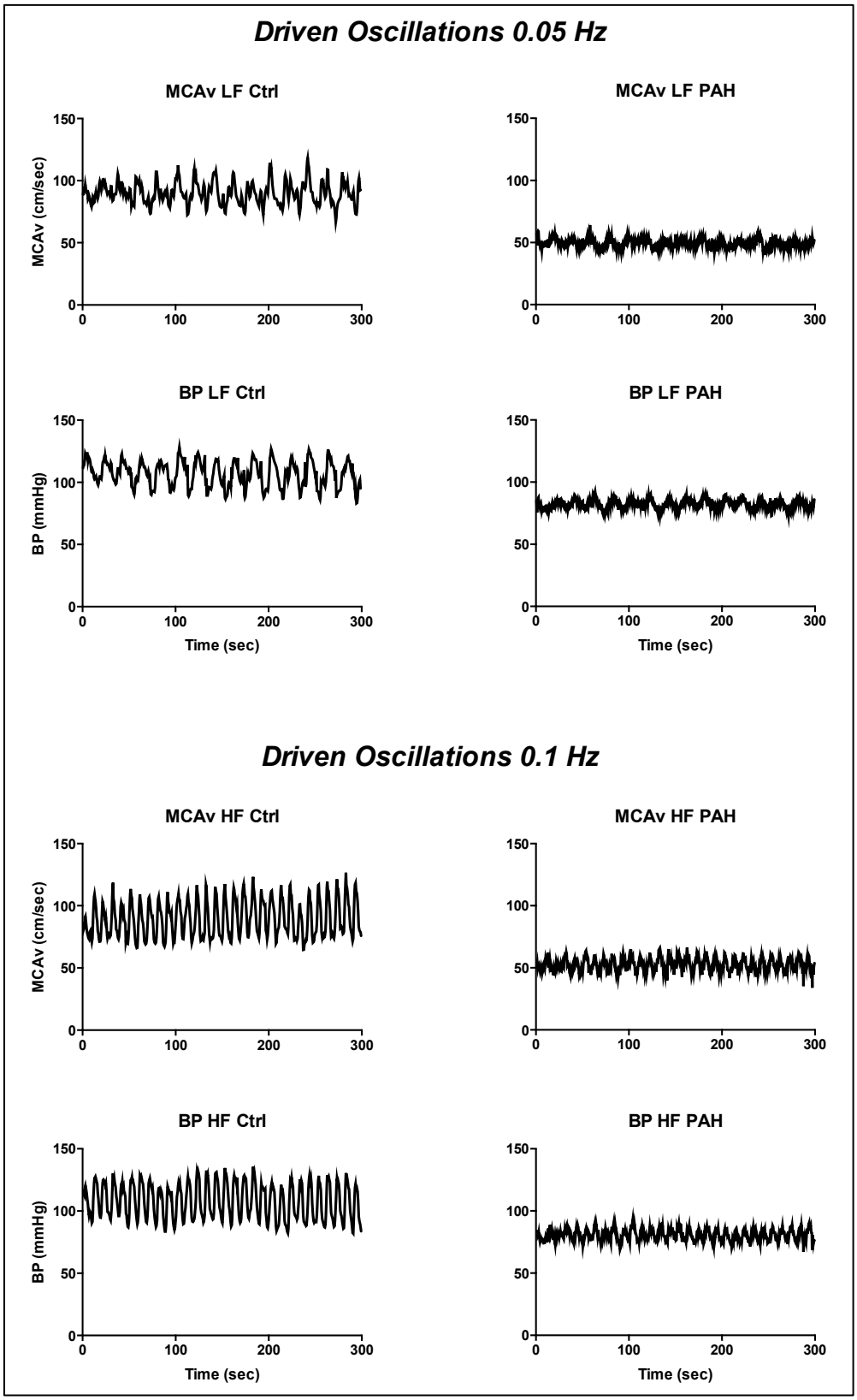


Figure S2. Representative resting trace.



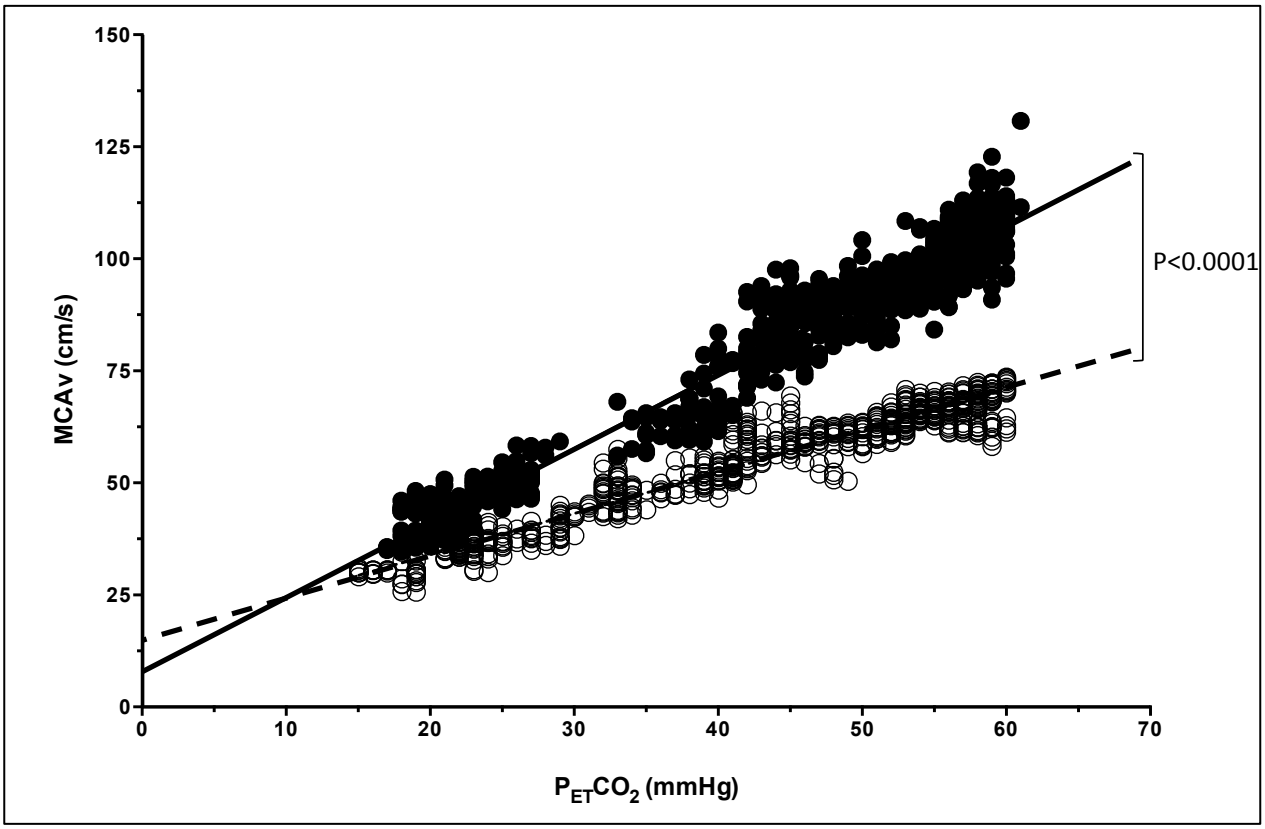
Data from one control subject and one PAH patient showing beat-by-beat variability in MCAv and BP in the time domain for the seating rest period of 5 minutes. Abbreviations are: MCAv: middle cereal artery velocity; BP: blood pressure; Ctrl: control; PAH: pulmonary arterial hypertension.

Figure S3. Representative squat-stand trace.



Data from one control subject and one PAH patient showing beat-by-beat variability in MCAv and BP in the time domain for the squat-stand maneuvers driven oscillations at 0.05 Hz and 0.1 Hz. Abbreviations are: MCAv: middle cereal artery velocity; BP: blood pressure; Ctrl: control; PAH: pulmonary arterial hypertension.

Figure S4. Absolute cerebrovascular reactivity to CO_2.



Poon-adjusted [4] absolute cerebrovascular reactivity to CO_2 analysis demonstrated that PAH patients (open circles) had blunted reactivity, as MCAv being consistently lower at iso-$P_{ET}CO_2$ throughout the physiologic range of $P_{ET}CO_2$ (0.91 (0.06) vs 1.66 (0.03) cm/s/mmHg; $p<0.0001$), compared to controls (closed circles). Abbreviations are: MCAv: middle cerebral artery velocity; $P_{ET}CO_2$: end-tidal CO_2 partial pressure.

Supplemental References:

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