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Alveolar-Capillary Recruitment: The Relationship of Diffusion Capacity of the Lungs for Carbon Monoxide to Pulmonary Blood Flow in Response to Exercise in PAH Patients

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Abstract

Exercise provokes interaction between cardiac and pulmonary systems allowing for the detection of underlying dysfunction.

Purpose: Evaluate if limitations in alveolar-capillary expansion (DLCO/Qc) during exercise exist in individuals with pulmonary arterial hypertension (PAH)

Methods: Sixteen individuals with mild to moderate Group 1 PAH and sixteen healthy subjects completed four 3-min stages of submaximal exercise. At rest and twice during each stage diffusion capacity of the lungs for carbon monoxide (DLCO) and pulmonary blood flow (Qc) were measured, with pulmonary gas exchange continuously recorded.

Results: There was a blunted drop in DLCO/Qc with the initiation of exercise for PAH participants driven by a reduced increase in Qc with a similar increase in DLCO (ΔDLCO/Qc: -0.52±0.80vs. -1.17±0.74, p=0.03; ΔQc: 1.5±1.4 vs. 2.4±0.7L/min, p=0.04; ΔDLCO: 1.2±1.4 vs. 1.9±2.4L/min/mmHg, p=0.31, PAH vs. Healthy respectively). This initial change in DLCO/Qc was related to the change in the gas exchange estimate of pulmonary capacitance for healthy individuals (r= -0.63, p=0.009), but related to the change in Qc for individuals with PAH (r= -0.64, p=0.009).

Conclusions: Despite effective medical management of mild-moderate disease, there appears to be a delayed alveolar-capillary expansion due to limitations in the recruitment/distention of pulmonary capillaries and these limitations present during exercise in otherwise stable patients suggest DLCO/Qc evaluation in response to exercise could be used as a non-invasive tool to track response to therapy and potentially detect PAH earlier.

Keywords: submaximal exercise; gas exchange capacitance; alveolar-capillary expansion; Group 1 PAH

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Acronym

DLCO – diffusion capacity of the lungs for carbon monoxide; DLCO/Qc – alveolar-capillary expansion; the relationship of diffusion capacity of the lungs for carbon monoxide to pulmonary blood flow; DLNO – diffusion capacity of the lungs for nitric oxide; ERA= endothelin receptor antagonist; GxCap – gas exchange estimate of pulmonary capacitance; HD= heart disease; HR – heart rate; IP = prostacyclin receptor agonist; O_2 Pulse - absolute VO_2 multiplied by heart rate, a surrogate for stroke volume; PAH – pulmonary arterial hypertension; PDE5= phosphodiesterase inhibitor; $PECO_2$ – mixed expired CO_2; PET-CO_2 – end-tidal CO_2; PGI2 = prostacyclin IV; Qc – pulmonary blood flow; RPE – a rating of perceived exertion; RVSP= right ventricular systolic pressure; SpO_2 – peripheral oxygen saturation; SV – stroke volume

Introduction

Pulmonary arterial hypertension (PAH) is a hemodynamically heterogeneous pathophysiological state with 1-2 cases per million people in the US and Europe per year [1]. It is a disease characterized by vascular obstruction and vasoconstriction leading to a progressive increase in pulmonary vascular resistance and eventually right ventricular failure [2]. Pulmonary hypertension is clinically defined as a mean pulmonary arterial pressure of more than 25 mm Hg at rest, or 30mmHg during exercise in the absence of pulmonary venous hypertension [1, 3]. Despite significant progress in our knowledge of PAH pathogenesis and therapeutic intervention, the key to successful treatment of the disease remains to be earlier intervention. PAH typically progresses slowly with most patients initially presenting with exertional dyspnea, chest pain, fatigue, or syncope that worsens over months to years; however, this early stage often remains undetected until pulmonary vascular obstruction and remodeling have already developed [4-6]. Consequently, patients are being diagnosed late which results in a poor prognosis with only 55% surviving the past three years [7]. Data from the national PAH registry in France reported an average delay of 27 months between the onset of symptoms and diagnosis, which at this time 75% of their patients already had severe clinical impairment (New York Heart Association functional class III or IV) [8]. Currently, there is no gold standard measurement to assess and track PAH severity. Non-invasive methods such as the six-minute walk test, WHO classification or echocardiography all lack sensitivity, accuracy, and/or are subjective or highly dependent on the technician or operator expertise [9-12]. On the other hand, the more invasive method of right-side cardiac catheterization pressure would be the gold standard, but its invasive nature prevents its use as a convenient trackable measure [9, 12], and measures are difficult with exercise and intrathoracic pressure changes with breathing tending to result in misdiagnoses that approach 20% [13]. The delay between symptom initiation and when PAH is confirmed by right heart

catheterization is generally more than two years [3, 14], leaving a limited window of opportunity for effective treatment. As such, there is a critical need for simple, inexpensive, and reliable techniques that allow for early detection and sequential monitoring of PAH in order to effectively evaluate, treat and manage patients, and ultimately prevent disease progression.

In his review of the best path forward to increase early detection of individuals with PAH, Dr. Palevsky highlighted that dyspnea upon exertion, although a nonspecific symptom, is the earliest the symptom that is present in the majority of patients [15], and previous research suggests that exercise-induced PAH is an early subclinical stage of PAH and is physiologically an intermediate in the transition from healthy to resting PAH [16]. We have previously demonstrated that gas exchange measures during a six-minute low-intensity step test can distinguish healthy and individuals with PAH, and further separate moderate PAH (WHO I and II) from those with more severe PAH (WHO III and IV) [17]. However, we also utilize a rebreathe method to quantify both diffusing capacity of the lungs for carbon monoxide (DLCO) as well as pulmonary blood flow (Qc), while maintaining a relatively normal and physiological breathing pattern [18, 19]. DLCO is highly dependent on both conductance across the alveolar the capillary membrane as well as pulmonary capillary blood volume, and therefore provides a good index of functional gas exchange surface area and enables an in-depth evaluation of the relationship of surface area expansion to blood flow (i.e., recruitment and distension) with exercise and thus may offer insight into limitations in this population. With exercise, cardiac output/pulmonary blood flow increases and there is subsequent recruitment and distention of pulmonary capillaries that increase diffusion surface area, and subsequently DLCO, and in healthy individuals both DLCO and blood flow increase linearly with exercise intensity with no evidence of an upper ceiling and the increase in Qc is one of the primary determinants of the increase in DLCO during exercise [20]. Previous work has demonstrated that resting DLCO alone tracks PAH severity [21], but evaluation of the change in DLCO and Qc to understand recruitment in response to exercise has not been evaluated in PAH. The importance of eval-

uating this ratio in disease has been proposed previously [22-24]. The purpose of this study was to determine the relationship of Qc, alveolar-capillary expansion (the relationship between DLCO and Qc: DLCO/Qc) and exercise workload in individuals with PAH and healthy matched control subjects. We hypothesize that the pulmonary vasoconstriction and remodeling that develop as PAH progresses will limit the changes in DLCO and Qc observed during exercise, altering the relationship between DLCO and Qc allowing it to be used to monitor changes in PAH severity with therapy and potentially serve as a sensitive parameter for detection of preclinical PAH in future work.

Methods

Participants and Informed Consent

Sixteen individuals diagnosed with Group 1 PAH and sixteen matched healthy control subjects were recruited to participate in the study. The individuals with PAH were all clinically diagnosed, on stable medications for at least three months, and were capable of performing the exercise. Clinical records were reviewed to document the most recent WHO classification made by the patient's cardiologist, echocardiography derived right ventricular systolic pressure (RVSP), peak tricuspid regurgitation velocity (TRV) and tricuspid annular plane systolic excursion (TAPSE) within six months of the study visit. The individuals with PAH were treated with endothelin receptor antagonist (ambrisentan, n=14); phosphodiesterase inhibitor (n=11); intravenous prostacyclin (n =4); soluble guanylate cyclase stimulator (n=1); prostacyclin receptor agonist (n=1). Healthy individuals had to be free of any cardiovascular or pulmonary disease and be a comparable age and sex match for recruited PAH individuals. There were seven healthy individuals on no medications, two on proton pump inhibitors, two taking thyroid medication, and one on a statin. All participants provided written informed consent to participate in the study, which had been approved by the Mayo Clinic Institutional Review Board. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Experimental overview

Participants completed a single visit consisting of an 11-min submaximal exercise bout on a semi-recumbent cycle ergometer (Corival, Lode, Netherlands). Following two minutes of resting data collection, the exercise protocol started with two minutes of unloaded cycling and three, three-minute stages with suggested workloads present for men (30-60-90 Watts) and women (20-40-60 Watts). However, the workloads were adjusted for an individual as necessary, such that the workloads were reduced, but always increased (minimum increase 5 Watts), so three stages were always completed with the goal of reaching a respiratory exchange ratio of ~around 1.00 and rating of the perceived level of exertion (RPE) ~12-14 by the final stage. Continuous monitoring included breath by breath respiratory gas exchange using a metabolic measurement system (Medgraphics, Saint Paul, MN), heart rate (HR) from a 12-lead ECG (GE Healthcare, Cardiosoft, Chicago, IL) and peripheral oxygen saturation (SpO_2) (Masimo, Radical-7, Irvine, CA) monitored continuously. During each stage, manual blood pressure was measured and participants RPE and level of dyspnea using the Borg and category ratio Borg Scale [25, 26]. As a secondary aim of the study, those who could and were willing were put on ambrisentan (Letaris®) for twelve weeks and then the submaximal exercise test was repeated. Unfortunately, only two patients were eligible for this, so this data is provided as case data only.

Measurement of Diffusion Capacity of the Lungs for Carbon Monoxide, Nitric Oxide and Assessment of Pulmonary Blood Flow

While seated in an upright position on the cycle ergometer resting DLCO and diffusion capacity of the lungs for nitric oxide (DLNO) and pulmonary blood flow (Qc) were measured simultaneously with the subjects using the rebreathing technique with a 5-liter anesthesia bag containing 0.7% acetylene, 9% helium, 0.3% carbon monoxide (C18O), 40 PPM NO (diluted immediately before the test in the bag from an 800 PPM gas mixture) and 35% O2, at a respiratory rate of 32 breaths/minute as described previously [27-29]. For all measurements, the bag volume matched the individual's tidal volume plus 500mL, with a minimum bag volume of 1000mL, to ensure the bag did not collapse during inhalation, but also did not cause an unnecessary excess of gas in the bag during the maneuver. At the end of a normal expiration (functional residual capacity), the subjects were switched into the rebreathe bag and instructed to nearly empty the bag with each breath for 8-10 consecutive breaths. The maneuver was performed in triplicate before exercise was initiated, and twice during each exercise stage.

The rate of disappearance of acetylene from the exhaled gas mixture during rebreathing is used to assess pulmonary blood flow. Since acetylene does not bind to hemoglobin,

the rate of disappearance of acetylene is limited primarily by the rate at which a new volume of blood is transported through the lungs. Because all the blood in the pulmonary circulation per minute is equal to the volume of blood in the systemic circulation per minute, the measure of the disappearance of acetylene provides a reliable measure of cardiac output and has previously been validated in our laboratory using direct Fick during exercise [30, 31]. Further, Hoeper *et al.* demonstrated acetylene rebreathing assessed Qc comparable to the Fick method in patients with PAH at least at rest [32]. Stroke volume was calculated by dividing measured Qc by the average HR during the maneuverer.

Gas exchange based surrogate of pulmonary vascular capacitance (Gxcap)

Pulmonary vascular capacitance, the ability to accept a volume of blood under a given pressure, is calculated by dividing stroke volume by pulmonary artery pulse pressure. In previous work, we have demonstrated in heart failure patients that non-invasive gas exchange measures of end tidal CO2 ($PETCO_2$) and oxygen pulse (O_2 Pulse = absolute VO_2 multiplied by heart rate) are correlated closely with measures of mean pulmonary artery pulse pressure and stroke volume [33]. Further O_2 pulse multiplied by $PETCO_2$, termed gas exchange capacitance (Gxcap) was highly correlated with pulmonary vascular capacitance and as such serves as a non-invasive surrogate of pulmonary vascular capacitance.

A gas exchange based marker of ventilation-perfusion mismatch ($PECO_2/PETCO_2$)

In the perfect lung where ventilation is uniform and perfectly matched to perfusion gas exchange will be most efficient, but in reality, since there is physiological dead space mixed expired CO_2 ($PECO_2$) is always lower than end-tidal CO_2 ($PETCO_2$) due to dead space dilution, such that the the ratio of $PECO_2$ to $PETCO_2$ is never zero. As such $PECO_2/PETCO_2$ provides a marker of ventilation to perfusion mismatch and $PECO_2$ will be reduced when there are impairments in ventilation, perfusion or both [34].

Statistical Analysis

The SPSS statistical software package (v25; SPSS, Chicago, IL) and GraphPad Prism (V8.0, GraphPad Software, San Diego, CA) was used for all analyses. Baseline, the final stage of the exercise, and change upon initiation of exercise comparisons between healthy and PAH groups were assessed using two-sided independent samples t-tests with a corrected p-value of 0.01 used to determine significance to account for multiple comparisons. The effect of exercise workload and condition were assessed using linear mixed model analysis, with a p-value of 0.05 used to identify a significant factor or interaction. Relationships between the change in the ratio of diffusion capacity of the lungs for carbon monoxide to pulmonary blood flow and the change in gas exchange surrogate of pulmonary capacitance or the change in pulmonary blood flow were evaluated using Pearson's correlation. All data are presented as mean±SD.

Results

The PAH population in this study was of mixed group 1 etiology, with almost half being idiopathic cases. In this group of individuals with PAH, half were being treated with dual therapy, with the remaining five and four on mono and triple therapy respectively (Table 1). As such, this was a group with established stable PAH, rather than a group with early/pre-clinical PAH. Additionally, the two cases discussed in the final section of the results provide an evaluation of the ability to monitor changes in PAH severity with therapy. Individuals with PAH were well matched to their healthy counterparts with no difference in anthropometric characteristics between groups (Table 1). The sub maximal exercise was a similar relative intensity for both groups with no difference in heart rate, oxygen consumption, respiratory exchange ratio or rating of perceived exertion at the final stage of exercise (Table 2). The absolute workload was higher for the healthy participants (p=0.003), and although the individuals with PAH tended to desaturate more than healthy controls this was not significant (p=0.11). The gas exchange response to the staged exercise was also not significantly different between PAH and Healthy groups (Table 3). Both DLCO and Qc increased in a similar fashion for both individuals with PAH and healthy controls (Figure 1A&B). With exercise individuals with PAH demonstrated a consistently lower gas exchange surrogate of pulmonary capacitance (GxCap) with the difference widening further during the final two stages of exercise (Figure 1C, workload x condition interaction p=0.007). There was also no significant difference between stroke volume or O_2 pulse (Figure 1D). The relationship between DLCO/Qc demonstrated a difference in the pattern of change with exercise (workload x condition interaction p=0.02), where there was a greater drop in DLCO/Qc with the initiation of exercise for healthy individuals compared to those with PAH (p=0.03, Table 4); although there was no significant difference between groups at any exercise stage (Figure 2).

When focusing on the change upon initiation of exercise, there was a reduced change in cardiac output, and spe-

cifically stroke volume, in those with PAH (p=0.04 and 0.02 respectively), whereas the increase in DLCO and GxCap were not different (p=0.31, Table 4). An evaluation of the potential factors influencing these differences, healthy and PAH groups showed opposite relationships. The drop in DLCO/Qc with the initiation of exercise for healthy individuals was related to the increase in GxCap (r= -0.76, p=0.001), but this was not true for those with PAH (r=0.06, p=0.84) (Figure 3A). For the PAH group, the drop in DLCO/Qc was related to the increase in Qc (r= -0.64, p=0.009), whereas there was no relationship for healthy individuals (r=-0.14, p=0.61) (Figure 3B).

Observational data: evaluation pre and post ambrisentan

Two individuals following the baseline visit were put on ambrisentan for twelve weeks and then the submaximal exercise was repeated. For the first patient ambrisentan was their only therapy and for the second patient ambrisentan became their second PAH medication. For these individuals, the evaluation of the change in response to the initiation of the exercise demonstrated that for both individuals the additional medication seemed to improve their response to exercise (Table 5). Specifically, for both individuals twelve-week post the change in DLCO/Qc increase due to greater changes in both DLCO and Qc and SV. For the individual who ambrisentan was a de novo medication, there was also a greater change in GxCap with exercise initiation. Further, for both patients unlike on their first exercise bout, there was no desaturation following the addition of ambrisentan.

Table 1: Subject Population	**Healthy**	**PAH**
n	16	16
Gender female	9 (56%)	9 (56%)
Age (years)	55±16	54±17
Height (cm)	169±11	170±10
Weight (kg)	75±14	79±21
BMI (kg/m²)	26±4	27±5
PAH Population		
RVSP (mmHg)		54±22
Peak TRV (m/s)		3.5±0.7
TAPSE (mm)		20±3
Functional Class (range 1-2)		**1.5±0.5**

Group 1 : 7 idiopathic
2 portopulmonary
2 scleroderma
2 lupus
1 congenital HD

Medications:
5 single therapy (ambrisentan or PDE5)
7 dual therapy (ERA & PDE5 or SGC)
4 triple therapy (PDE5, ERA and PGI2 or 1 familial

RVSP= right ventricular systolic pressure; TRV=tricuspid regurgitation velocity; TAPSE=tricuspid annular plane systolic excursion by M-mode; PDE5=phosphodiesterase inhibitor; ERA= endothelin receptor antagonist; PGI2 = prostacyclin IV; IP = prostacyclin receptor agonist; HD= heart disease

Table 2 Submaximal Exercise Final Stage	Healthy	PAH
Peak HR (bpm)	115±18	120±16
Percent of Predicted Max HR (%)	71±15	72±12
VO_2 peak (mL/min/kg)	14.7±5.2	11.9±2.7
ΔVO_2 from rest to the final stage (mL/min/kg)	10.8±4.7	7.8±2.4
Wattage final stage	71±16	53±17*
VO_2/W final stage	0.20±0.07	0.24±0.07
RER at peak	1.01±0.13	1.13±0.17
RPE	11.0±2.1	13.6±2.3
Desaturation (%)	0.9±1.5	2.6±3.8

VO_2 peak= peak oxygen consumption; RER= respiratory exchange ratio; RPE= rating of perceived exertion. * $p<0.01$ adjusted value for multiple comparisons in independent samples t-test Healthy vs. PAH

Baseline			Stage 1 (unloaded)		Stage 2		Stage 3		Stage 4		Recovery	
Healthy		PAH	Healthy	PAH	Healthy	PAH	Healthy	PAH	Healthy	PAH	Healthy	PAH
VO_2 (mL/min/Kg)	3.9±1.1	4.5±1.7	8.2±2.4	8.0±2.1	9.8±2.6	9.7±1.8	12.0±3.7	11.0±2.3	14.6±5.3	12.1±3.0	10.2±3.5	10.3±3.2
Vt (mL)	755±436	831±258	1002±342	1014±284	1104±310	1242±304	1272±379	1396±333	1468±469	1466±367	1233±417	1362±308
RR (bpm)	17±4	19±6	24±7	25±6	25±3	27±6	26±5	28±4	28±7	31±5	28±5	30±6
VE (L/min)	11.8±5.4	15.4±7.8	22.3±6.9	24.5±7.5	26.2±6.7	32.3±7.1	31.0±6.8	38.8±10.0	40.0±11.0	45.1±12.4	33.8±11.7	40.5±12.1
VE/VCO_2	45±8	50±10	46±24	48±7	41±9	45±6	40±17	44±7	36±10	45±10	40±11	45±9
$PETCO_2$ (mmHg)	33±6	29±6	33±4	29±5	34±4	30±6	35±4	31±68	36±4	31±7	35±4	31±7
$PECO_2$ (mmHg)	20±4	19±4	22±4	18±3	23±4	21±4	24±5	22±5	26±6	22±5	24±5	21±5
$PECO_2$/ $PETCO_2$	0.62±0.07	0.66±0.09	0.67±0.09	0.68±0.06	0.68±0.10	0.71±0.06	0.69±0.11	0.71±0.07	0.70±0.13	0.71±0.09	0.69±0.12	0.71±0.09
SpO_2 (%)	100±0	98±2	99±1	97±3	99±1	97±3	99±2	96±4	99±2	96±4	99±1	98±3
ΔSpO2 (%)	-	-	-0.4±0.9	-0.8±2.0	-0.5±0.8	-1.4±2.2	-0.8±1.4	-1.8±2.6	-0.9±1.5	-2.6±3.8	-0.3±0.5	-0.7±2.6
SBP (mmHg)	122±10	119±12	130±17	131±16	139±19	136±21	146±22	149±22	162±27	159±29	133±16	135±23
DBP (mmHg)	76±5	72±9	78±6	74±9	77±6	75±10	79±9	77±10	80±10	80±11	76±6	72±10
HR (bpm)	69±10	78±10	83±17	93±11	90±15	100±12	100±15	110±13	115±17	119±16	85±17	94±15
DM												
(L/min/mmHg)	24.9±11.4	21.1±9.4	27.4±11.2	21.8±9.4	27.4±11.4	23.3±9.3	28.1±12.0	24.5±10.0	31.0±13.1	24.6±10.2	28.4±12.1	23.1±12.1
Vc (mL)	89.8±28.6	82.7±58.2	100.3±33.7	100.3±75.1	110.8±57.8	106.4±84.5	112.4±34.9	120.8±99.3	118.6±34.8	13.8±64.3	95.7±30.4	01.7±89.2
DM/Vc	0.30±0.13	0.31±0.13	0.29±0.11	0.28±0.13	0.29±0.11	0.28±0.11	0.27±0.11	0.26±0.12	0.28±0.12	0.26±0.13	0.31±0.11	0.31±0.15

Table 3: Change in Gas Exchange, Hemodynamic Parameters and Components of DLCO in Response to Submaximal Exercise.

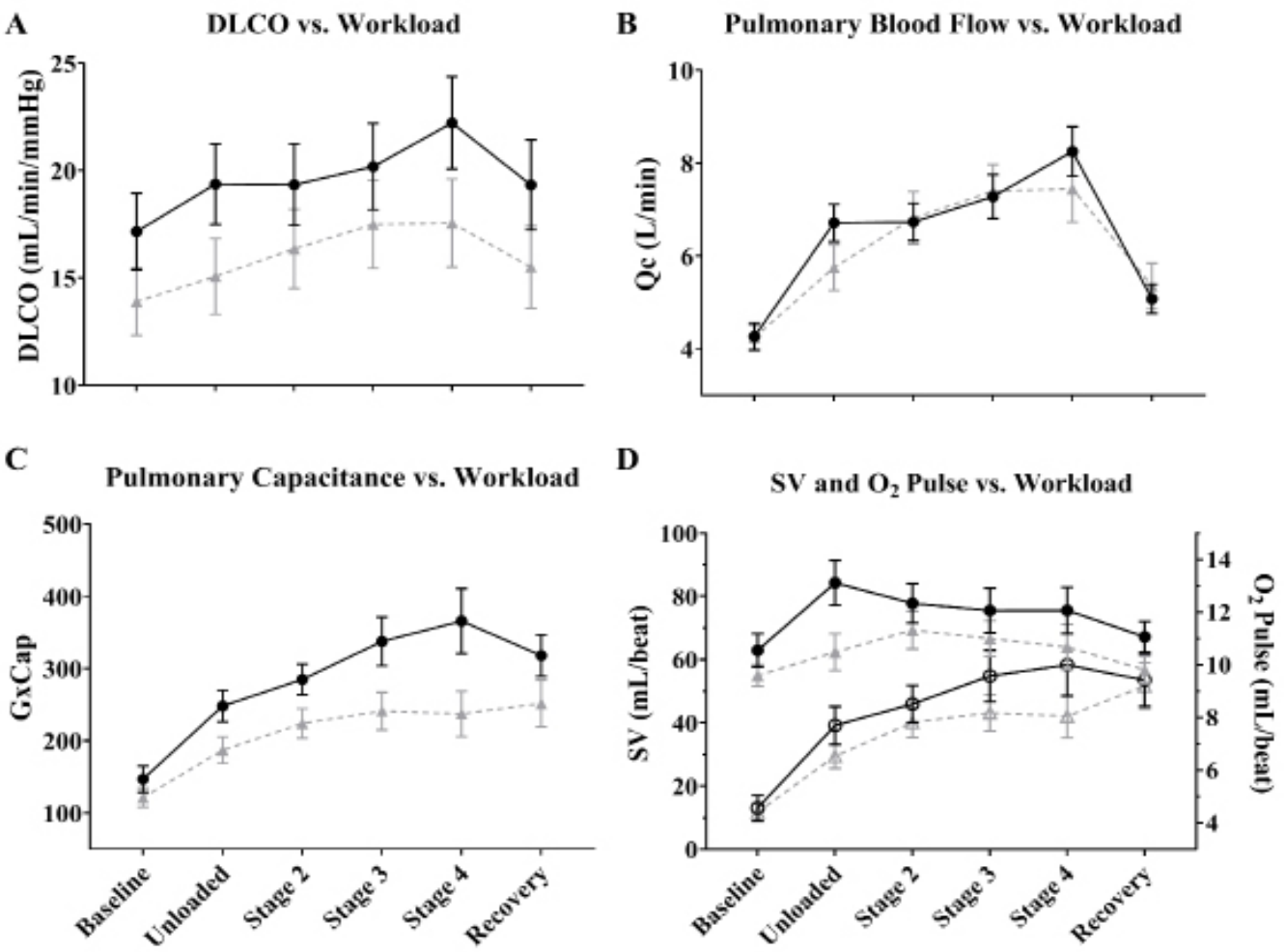

Figure 1: Change in Diffusion capacity of the lungs for carbon monoxide (DLCO, Panel A), Pulmonary blood flow (Qc, Panel B), Gas exchange surrogate of pulmonary capacitance (GxCap, Panel C) and stroke volume and oxygen pulse (SV and O_2 Pulse, Panel D) from rest through unloaded and three 3 minute increasing workloads of submaximal exercise and recovery. Comparing individuals with Group 1 PAH (grey triangles and dashed line) to healthy controls (black circles and solid line). Oxygen pulse is represented by open grey triangles for PAH and open black circles for healthy controls. There was a significant workload x condition interaction effect for GxCap, p=0.007.

Change upon initiation of exercise	Healthy	PAH	P-value
ΔDLCO (rest to stage 1)	1.9±2.4	1.2±1.4	0.31
ΔQc (rest to stage 1)	2.4±0.7	1.5±1.4	0.04
ΔSV (rest to stage 1)	20±13	7±15	0.02
ΔHR (rest to stage 1)	15±10	14±10	0.70
ΔGxCap (rest to stage 1)	103±61	78±53	0.22
ΔDLCO/Qc (rest to stage 1)	-1.17±0.74	-0.52±0.80	0.03

Table 4: Δ=change from rest to stage 1; DLCO= diffusion capacity of the lungs for carbon monoxide; Qc= pulmonary blood flow; SV= stroke volume; GxCap= gas exchange derived pulmonary capacitance; DLCO/Qc= ratio of DLCO to Qc. P-value independent samples t-test Healthy vs. PAH

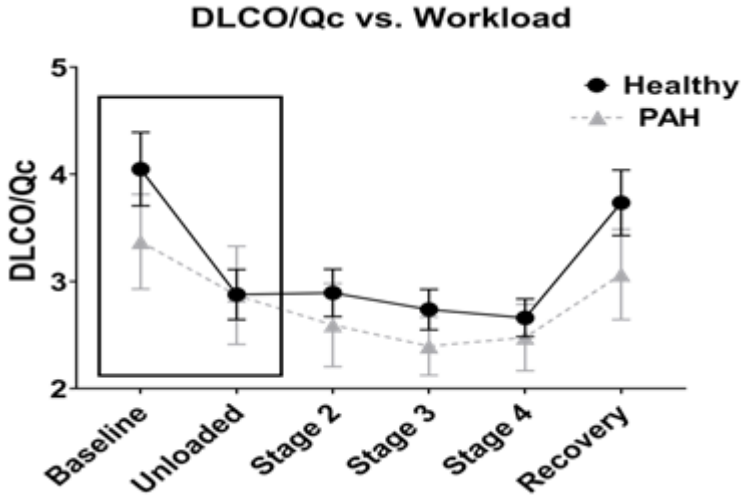

Figure 2 The Relationship of Cardiac Output to Alveolar-capillary Expansion in Response to Submaximal Exercise

Change in ratio of diffusion capacity of the lungs for carbon monoxide to pulmonary blood flow (DLCO/Qc) from rest through unloaded and 3 3 minute increasing workloads of submaximal exercise and recovery. Comparing individuals with Group 1 PAH (grey triangles and dashed line) to healthy controls (black circles and solid line). Workload x condition interaction effect p=0.02 for any of these variables. Black rectangle highlight the change from baseline to unloaded cycling (i.e., the initiation of exercise), for further analysis of this period see data in Table 4 and Figure 3.

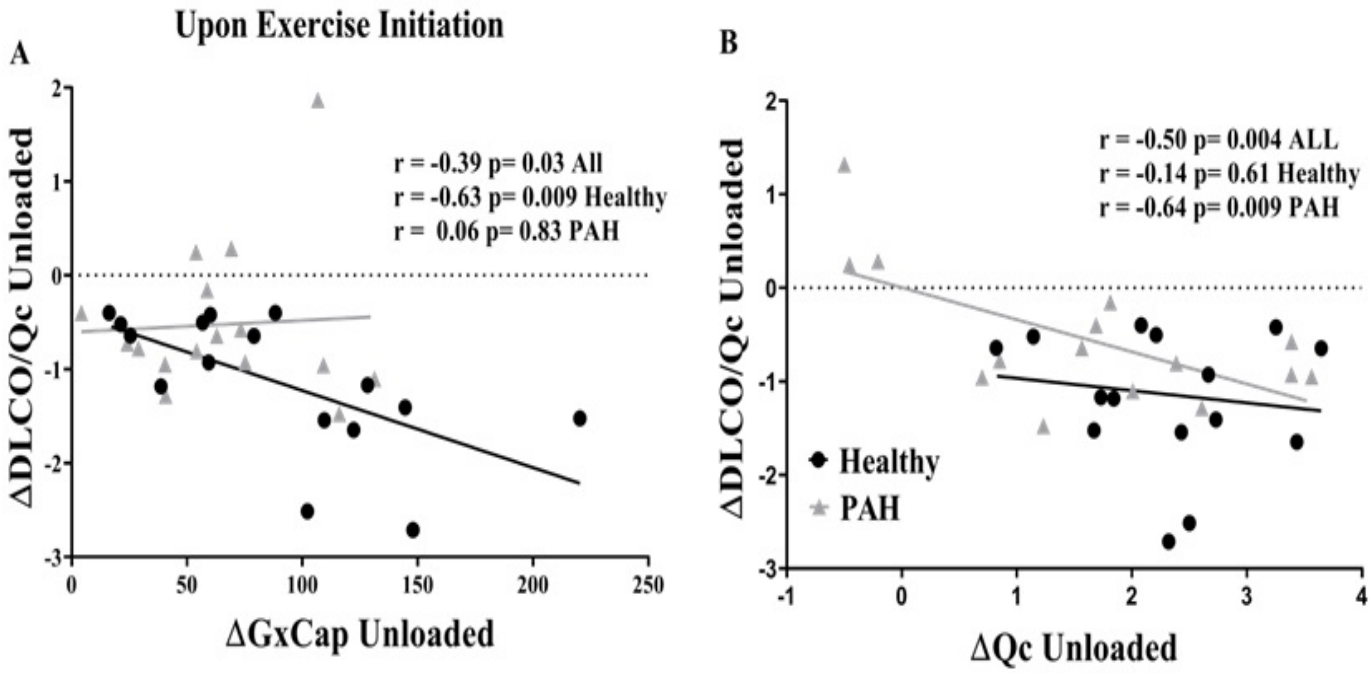

Figure 3 Factors influencing Alveolar-capillary Expansion upon Exercise Initiation

The relationship between the change in ratio of diffusion capacity of the lungs for carbon monoxide to pulmonary blood flow (DLCO/Qc) and the change in gas exchange surrogate of pulmonary capacitance (GxCap, Panel A) and the change in pulmonary blood flow (Qc, Panel B) from rest to unloaded cycling. Individuals with PAH represented by grey triangles and grey trend line, and healthy controls represented by black circles and black trend line. Pearson correlation results and p-values for the entire group, and PAH and Healthy conditions separately are provided on the panels.

Table 5 Pre to Post ambrisentan Change upon initiation of exercise	Patient 1: De novo Med		Patient 2: Second Med	
Addition of ambrisentan 12 weeks	**Pre**	**Post**	**Pre**	**Post**
ΔDLCO (mmHg)	-0.39	3.97	0.5	0.95
ΔQc (L/min)	-0.46	2.9	1.56	4.14
ΔSV (ml/beat)	-13.6	29.4	-0.5	29.7
ΔGxCap	54	98	63	62
ΔDLCO/Qc	0.25	-0.88	-0.64	-0.93
Peak Desaturation (%)	-3	0	-2	0

Δ=change from rest to stage 1; DLCO= diffusion capacity of the lungs for carbon monoxide; Qc= pulmonary blood flow; SV= stroke volume; GxCap= gas exchange derived pulmonary capacitance; DLCO/Qc= ratio of DLCO to Qc.

Discussion

In this study, we demonstrated that there was a blunted alveolar-capillary expansion (the relationship between DLCO and Qc) with the initiation of exercise for individuals with PAH due to a reduced change in Qc. Further, when evaluating what is driving this difference, the ability to expand and recruit capillaries was related to flow for those with PAH, but capacitance for healthy individuals. Additionally, we provided preliminary data demonstrating the potential utility of submaximal exercise and evaluation of alveolar-capillary expansion for monitoring a patient's response to new or additional treatments. The results of this study build upon previous work suggesting exercise is a valuable tool for interrogating the pulmonary vasculature and highlights the utility of evaluating the balance between blood flow and gas exchange surface area which can detect differences even when gas exchange alone may not distinguish PAH from healthy.

The ability to augment cardiac output/pulmonary blood flow is a critical player in the progression of symptoms developing in PAH due to hemodynamic dysregulation. Such that during the early stages of PAH, while pulmonary pressure is rising, cardiac output/pulmonary blood flow can be fairly maintained at rest, but when cardiac output cannot rise appropriately with exercise, symptoms develop [9]. Normally with exercise, the pulmonary vasculature distends as the primary means to accommodate this new flow initially, this only increases DLCO moderately. The recruitment of new capillaries allows for a greater change in surface area than can be achieved by distension alone, so it is normal for the DLCO/Qc relationship to drop initially due to a greater increase in pulmonary blood flow than there is a rise in DLCO [35-37].

The observation that upon initiation of exercise the change in DLCO/Qc related to pulmonary capacitance (GxCap) in healthy participants, but in those with PAH the change in DLCO/Qc was related to flow, highlights PAH pathophysiology. The fact that the change in DLCO/Qc was not related to pulmonary capacitance suggests a reduced ability to dilate in those with PAH. Since PAH is a disease which leads to increased pulmonary vascular resistance and loss of pulmonary vasodilator response to exercise, the results of this study demonstrate that this can be detected, sometimes even without observing signs of inefficient lung gas exchange [38]. A reduced ability to dilate and accept the increased blood flow along with higher vascular resistance necessitates a larger increase in flow to generate enough pressure to 'pop open' and recruit new blood vessels. Individuals with PAH demonstrated a reduced increase in Qc upon the initiation of exercise, but with the additional workload, the PAH individuals' response was similar to their healthy counterparts where both showed a plateau in the subsequent exercise stages as DLCO was increasing at a similar rate to Qc. As such, the blunted increase in Qc resulted in less of a drop in DLCO/Qc initially. This observation follows the pathophysiology of the condition where the inability of the

RV to adequately increase pulmonary blood flow in response to increased oxygen demand during exercise, or when at rest as severity increases, results in the symptoms of dyspnea and fatigue [38, 39]. However, in this group with established

PAH, there was not a complete inability, only a delay suggesting greater pre-load dependence [40]. Although this is only a temporary delay in the alveolar-capillary expansion, it is relevant to the PAH individuals' life as this "exercise initiation phase" would be encountered on a daily basis as they complete day today activities such as standing up from the couch, getting out of bed, walking to the mailbox, etc.; they may not participate in longer duration submaximal exercise daily, but they do initiate activity from rest to low activity and as such would experience this shortfall repeatedly.

In this group of mild to moderate PAH, the characteristic gas exchange metrics which have been shown to differentiate between PAH and healthy controls in previous studies were not different, albeit with some trends observed that coincide with earlier reports [17, 34, 41]. For example, $PETCO_2$ did not fall as is characteristic of more severe or uncontrolled PAH [34] but showed a limited increase in contrast to the healthy subjects where $PETCO_2$ increased with increasing work which has been demonstrated before [17]. VE/VCO_2 was higher at rest and throughout the exercise bout but did fall with exercise in a pattern similar to the healthy participants, in contrast to what has been found previously [17]. However, this group of individuals with PAH demonstrated the typical desaturation with exercise that has been described previously [17]. The fact that the current PAH subject population was of higher and less severe functional class compared to the PAH population recruited in earlier work (Current: RVSP 54; WHO functional class average 1.5 vs. Woods: RVSP 76; WHO 2.2) than the group of patients in previous studies may be one explanation for the inability for gas exchange to distinguish between the two groups. Another possible explanation is there have been major improvements in the medical care of these patients over the past nine years. In the present study, 68% of patients were on dual or triple therapy, compared to the population studied by Woods, *et al.* which had 65% on dual therapy only. Current therapies for PAH target abnormalities occurring in one of the three pathways: prostacyclin, nitric oxide or endothelin, with the targeting of no one pathway proving effective in all patients. This suggests that no one pathway dominates pathogenesis for all PAH patients, but can vary patient to patient [42]. As such, use of combination therapy allows for the targeting of multiple pathways, potentially increasing overall therapeutic effect. There have been multiple clinical trials of sequential therapy and a few initial combination trials with varying results some showing improved exercise capacity, functional class and hemodynamics [43-47], and recent meta-analysis have suggested that combination therapy can significantly reduce the risk of clinical worsening [48-51], such that with effective medical management the current data suggests PAH patients can retain a greater tolerance to exercise, as evidenced by near-normal pulmonary gas exchange metrics.

Finally, previous work has shown gas exchange can reveal changes in disease status [52], a finding that was also observed in the two patients who were re-examined after beginning ambrisentan treatment. In these patients, improvements in condition with the addition of ambrisentan were observed, specifically looking at the response to the initiation of exercise and evaluation of DLCO/Qc relationship and associated parameters. Although this observational data is only in a two patients, it highlights the potential of utilizing submaximal exercise and evaluation of alveolar-capillary expansion in response to exercise as a means to evaluate the effectiveness of new or additional medication simply and non-invasively and warrants further evaluation in future studies.

Limitations

In this study, we recruited a population of Group 1 PAH patients of mixed etiology and as such the sample size could be considered relatively small. However, the fact that differences could be found within this heterogonous population speaks to the sensitivity and wide-ranging application of evaluation of alveolar-capillary expansion in response to exercise in PAH. Additionally, invasive hemodynamics were not completed as a part of this study, so baseline characteristics relied on what had been performed clinically to classify disease severity. Since the subject population was primarily stable PAH patients, a right heart catheterization had not been done within six months of the study visit for all subjects. Instead, all subjects were being monitored by echocardiography and all had had an echo within three months of their study visit, so RVSP, TRV and TAPSE, along with the most recent WHO classification made by the patient's primary cardiologist's classification within the last 3 months was used to describe the severity of the group. As such, conclusive classification of the disease severity of these patients is not possible, instead this is data provides pilot data that to support further research to continue the evaluation of this potential tool. Future work in a population of a group of classical "borderline" PAH patients with concurrent invasive hemodynamic data is needed to make conclusions about whether the DLCO/Qc exercise response can be used as a non-invasive tool to detect undiagnosed mild PAH earlier, but this study does provide early data that suggests this could be possible as even in a a small sample of mixed etiology we could detect a differ-

ence in the alveolar-capillary response to the initiation of exercise. Finally, we would have liked have recruited more subjects to evaluate changes following medication changes, but we were only able to recruit patients who were not already on an ERA and could be put on ambrisentan which was a very small group within our an available clinical population so future work is still needed to determine if the evaluation of DLCO/Qc with exercise has application as a means of determining the efficacy of new or additional medication(s).

Conclusion

With the initiation of exercise, there was a reduced change in DLCO/Qc relationship for PAH patients are driven primarily by less of a change in Qc. PAH individuals also demonstrated a blunted the initial increase in pulmonary capacitance (GxCap) with exercise. Medical management appears to be effective in individuals with the stable mild-moderate disease, but they still appear to be a delay in recruitment/distention of pulmonary capillaries which may have the potential to be used as a tool to detect undiagnosed mild PAH earlier and in determining if a new medication is proving beneficial.

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Disclosure Statement

The authors declare that they have no competing interests.

Author contributions

BDJ, CMW conceived and designed the experiments. CMW, GMS, GP, and BSC performed the experiments. CMW analyzed the results. All authors contributed to writing this paper.

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