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ORIGINAL ARTICLE

Unexplained exertional intolerance associated with impaired systemic oxygen extraction

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Abstract

Purpose The clinical investigation of exertional intolerance generally focuses on cardiopulmonary diseases, while peripheral factors are often overlooked. We hypothesize that a subset of patients exists whose predominant exercise limitation is due to abnormal systemic oxygen extraction (SOE).

Methods We reviewed invasive cardiopulmonary exercise test (iCPET) results of 313 consecutive patients presenting with unexplained exertional intolerance. An exercise limit due to poor SOE was defined as peak exercise $(Ca\text{-}vO_2)/[Hb] \leq 0.8$ and $VO_{2max} < 80\%$ predicted in the absence of a cardiac or pulmonary mechanical limit. Those with peak $(Ca\text{-}vO_2)/[Hb] > 0.8$, $VO_{2max} \geq 80\%$, and no cardiac or pulmonary limit were considered otherwise normal. The otherwise normal group was divided into hyperventilators (HV) and normals (NL). Hyperventilation was defined as peak $PaCO_2 < [1.5 \times HCO_3 + 6]$.

Results Prevalence of impaired SOE as the sole cause of exertional intolerance was 12.5% (32/257). At peak exercise, poor SOE and HV had less acidemic arterial blood compared to NL (pHa = 7.39 ± 0.05 vs. 7.38 ± 0.05 vs. 7.32 ± 0.02, $p < 0.001$), which was explained by relative hypocapnia ($PaCO_2 = 29.9 \pm 5.4$ mmHg vs. 31.6 ± 5.4 vs. 37.5 ± 3.4, $p < 0.001$). For a subset of poor SOE, this relative alkalemia, also seen in mixed venous blood, was associated with a normal PvO_2 nadir (28 ± 2 mmHg vs. 26 ± 4, $p = 0.627$) but increased SvO_2 at peak exercise (44.1 ± 5.2% vs. 31.4 ± 7.0, $p < 0.001$).

Conclusions We identified a cohort of patients whose exercise limitation is due only to systemic oxygen extraction, due to either an intrinsic abnormality of skeletal muscle mitochondrion, limb muscle microcirculatory dysregulation, or hyperventilation and left shift the oxyhemoglobin dissociation curve.

Keywords Cardiopulmonary exercise testing · Exertional intolerance · Poor systemic oxygen extraction · Hyperventilation · Chronic fatigue syndrome

Abbreviations

BWH	Brigham and Women's Hospital
CaO_2	Oxygen content in arterial blood
$Ca\text{-}vO_2$	Difference between oxygen content in arterial and venous blood
CI	Cardiac index
CvO_2	Oxygen content in mixed venous blood

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DBP	Diastolic blood pressure
[Hb]	Hemoglobin concentration
HCO_3	Bicarbonate
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HR	Heart rate
HV	Hyperventilators
iCPET	Invasive cardiopulmonary exercise testing
LVEF	Left ventricular ejection fraction
MAP	Mean arterial pressure
MM	Mitochondrial myopathies
mPAP	Mean pulmonary arterial pressure
NL	Normal subjects
$PaCO_2$	Partial pressure of carbon dioxide in arterial blood
PAH	Pulmonary arterial hypertension
PaO_2	Partial pressure of oxygen in arterial blood
PCWP	Pulmonary capillary wedge pressure
PH	Pulmonary hypertension
pHa	Arterial pH
pHv	Venous pH
PML	Pulmonary mechanical limit
$PvCO_2$	Partial pressure of carbon dioxide in venous blood
PvO_2	Partial pressure of oxygen in venous blood
PVR	Pulmonary vascular resistance
Q_t	Cardiac output
Q_{tmax}	Cardiac output at maximum exercise
RAP	Right atrial pressure
RER	Respiratory exchange ratio
RR	Respiratory rate
SaO_2	Oxygen saturation in arterial blood
SBP	Systolic blood pressure
SOE	Systemic oxygen extraction
SOE_H	Poor SOE group with high PvO_2
SOEL	Poor SOE group with low PvO2
SVR	Systemic vascular resistance
SvO_2	Oxygen saturation in mixed venous blood
VCO_2	Carbon dioxide output
V_E	Minute ventilation
V_{Emax}	Minute ventilation at peak exercise
VO_2	Oxygen uptake
VO_{2max}	Maximum oxygen uptake

Introduction

An exercise limit can result from dysfunction of the lungs, heart, skeletal muscle, and nervous system, alone or in combination. When the cause of exertional intolerance is not understood after conventional testing, invasive cardiopulmonary exercise testing (iCPET) can be diagnostic and often points to previously unrecognized disorders of the heart or lungs (Borlaug et al. 2010; Maron et al. 2013; Santos et al. 2015; Tolle et al. 2008b). Peripheral limits to exertion, including impaired skeletal muscle oxidative phosphorylation, are more difficult to recognize and are less well studied (Bravo et al. 2012; Elliot et al. 1989; Flaherty et al. 2001; Gimenes et al. 2011; Heinicke et al. 2011; Jensen et al. 2002; Lindholm et al. 2004; Meulemans et al. 2007; Taivassalo et al. 2002, 2003; Tarnopolsky 2004; Tarnopolsky and Raha 2005).

Maximum oxygen uptake (VO_{2max}) quantifies the degree of exercise impairment in a symptomatic patient. According to the Fick equation, the product of the cardiac output (Q_t) and the difference between arterial and venous oxygen content ($Ca\text{-}vO_2$) is equal to oxygen uptake (VO_2). In the absence of a pulmonary mechanical limit, the Fick principal dictates that a depressed VO_{2max} can be explained by inadequate oxygen delivery or its uptake and utilization by the exercising limb skeletal muscle. Therefore, at maximum exercise, impaired oxygen delivery or extraction could cause exertional intolerance, including fatigue and dyspnea. For example, we have recently shown exercise-induced pulmonary arterial hypertension (PAH) (Tolle et al. 2008b) and pulmonary venous hypertension (Santos et al. 2015) have inadequate Q_t augmentation at peak exercise resulting in decreased VO_{2max}.

Abnormal peripheral oxygen extraction has been described in PAH (Tolle et al. 2008a), heart failure with reduced ejection fraction (Wasserman et al. 2012), and heart failure preserved ejection fraction (HFpEF) (Abudiab et al. 2013; Bhella et al. 2011; Dhakal et al. 2015; Haykowsky et al. 2011; Tolle et al. 2008a). It is also the exercise hallmark of the mitochondrial myopathies (MM), both genetic and secondary. While often thought of as a pediatric disease, MM due to confirmed mitochondrial DNA mutations can penetrate in adulthood and are estimated to have a prevalence of about 1 in 15,000 for adults aged 16–60 years (Chinnery et al. 2000). Acquired, secondary MM, due to infection or autoimmune processes for example, is likely even more common. Indirect measures during exercise testing in adult patients with MM have suggested that these patients have decreased VO_{2max} due to impaired oxygen extraction, manifested by decreased $Ca\text{-}vO_2$ with preserved Q_t at maximum exertion (Q_{tmax}) (Bravo et al. 2012; Flaherty et al. 2001; Gimenes et al. 2011; Heinicke et al. 2011; Lindholm et al. 2004; Taivassalo et al. 2002, 2003).

No study to date has directly identified impaired systemic oxygen extraction (SOE) as a sole source of exercise intolerance in a heterogeneous patient population with unexplained exertional intolerance. In the current study using directly measured Fick principle variables, we hypothesize that a significant proportion of patients will have poor SOE as the only identifiable etiology of their exertional intolerance.

Methods

Subjects

Results from iCPETs performed on 313 consecutive patients presenting to Brigham and Women's Hospital (BWH) between March 2011 and October 2013 for unexplained exertional intolerance, including those with suspected mitochondrial disease, were analyzed. All tests were performed for clinical indications, and the data were retrospectively reviewed. Medical records were reviewed for demographic, anthropometric, and clinical baseline characteristics. Available records from the poor SOE group (defined below) were further reviewed for additional clinical characteristics pertinent to any diagnosis related to poor exertional intolerance. Hemodynamic and metabolic data obtained from the pulmonary and radial arterial catheters as well as respiratory data measured from rest through peak exercise during iCPET were also collected. The study was approved by the Partners Human Research Committee (IRB #2011P000272), and written consent was obtained.

Invasive cardiopulmonary exercise testing

Our protocol for iCPET has been previously described (Maron et al. 2013). Briefly, a pulmonary artery catheter was placed in the BWH cardiac catheterization lab using standard procedure via the internal jugular vein with ultrasound and fluoroscopic guidance. The pulmonary artery catheter was a flow-directed, balloon-tipped, 4-port pacing pulmonary arterial catheter (Edwards Lifesciences, Irvine, CA). An arterial line was inserted into the radial artery using a 20-gauge angiocatheter or 5-French sheath.

All exercise tests were performed in the BWH cardiopulmonary exercise laboratory located adjacent to the cardiac catheterization laboratory. All subjects completed a single bout of incremental cycling to exhaustion on an upright ergometer (Medgraphics Corival Cycle Ergometer, Medical Graphics Corp, St. Paul, MN). The tests were performed with the subjects breathing room air. At least 2 min of rest was followed by 3 min of unloaded cycling at 40–60 revolutions per minute. Work was then continuously increased using a ramp protocol by 5, 10, 15, or 20 watts per minute based on historic exercise tolerance in the field. After reaching a symptom-limited maximum, the subject entered a recovery period.

Pulmonary gas exchange was measured breath-by-breath, and ventilation monitored with a commercially available metabolic cart (MedGraphics Ultima, Medical Graphics Corp., St. Paul, MN). Heart rate (HR), systemic systolic blood pressure (SBP), systemic diastolic blood pressure (DBP), right atrial (RAP), right ventricular, and pulmonary artery (PAP) pressures were measured continuously using a Phillips Xper Cardio Physiomonitoring System (Andover, MA) that was calibrated, leveled, and zeroed at the level of the right atrium before each study. Each minute at rest and during exercise, pulmonary capillary wedge pressure (PCWP) was measured during a passive exhalation (or when not possible, calculated as an electronic mean over the respiratory cycle) (Boerrigter et al. 2014), and a 12-lead electrocardiogram was obtained. One-milliliter blood samples were simultaneously drawn from the radial arterial catheter and distal port of the non-wedged pulmonary arterial catheter once every minute during rest, during the last 15-s of each minute during exercise, and once every minute during a 2-min recovery period immediately following peak exercise. Systemic arterial and mixed venous blood samples were analyzed at 37 °C (27) for partial pressure of oxygen (PaO_2 or PvO_2), partial pressure of carbon dioxide ($PaCO_2$ or $PvCO_2$), pH (Model 1620, Instrumentation Laboratories, Lexington, MA), bicarbonate concentration (HCO_3), hemoglobin concentration ([Hb]), oxygen saturation (SaO_2 or SvO_2) (Model 482, Instrumentation Laboratories), and oxygen content (CaO_2 or CvO_2) by co-oximetry. Systemic arterial blood samples were also analyzed for lactate concentration.

Data analysis

Ventilatory and pulmonary gas exchange data were averaged over the final 30-s interval of the 2-min rest period and averaged over contiguous 30-s intervals during exercise. VO_{2max} was defined as the highest 30-s averaged VO_2 during the last minute of the symptom-limited exercise test. Predicted values of VO_{2max} were those of Hansen and colleagues (1984) and were controlled for age, sex, and height. Q_t was calculated from the direct Fick method ($Q_t = VO_2/([Ca\text{-}vO_2])$, and predicted Q_{tmax} was calculated from predicted VO_{2max} and an assumed maximal $Ca\text{-}vO_2$ equivalent to a normal [Hb] (14 g/dL) for healthy subjects (Wasserman et al. 2012). Systemic vascular resistance (SVR) was calculated as $[80 \times (MAP\text{-}RAP)]/(Q_t)$, where MAP is the mean arterial pressure. Pulmonary vascular resistance (PVR) was calculated as $[80 \times (mPAP\text{-}PCWP)]/Q_t$, where mPAP is the mean PAP. The respiratory exchange ratio (RER) was calculated from $RER = VCO_2/VO_2$ averaged over contiguous 30-s intervals, where VCO_2 is carbon dioxide output.

Subjects were excluded if they had submaximal exercise testing (defined by RER < 1.0 and peak HR < 80% of predicted) (Pinkstaff et al. 2010), moderate or severe valvular heart disease, or left ventricle ejection fraction (LVEF) < 50% from a contemporary resting transthoracic

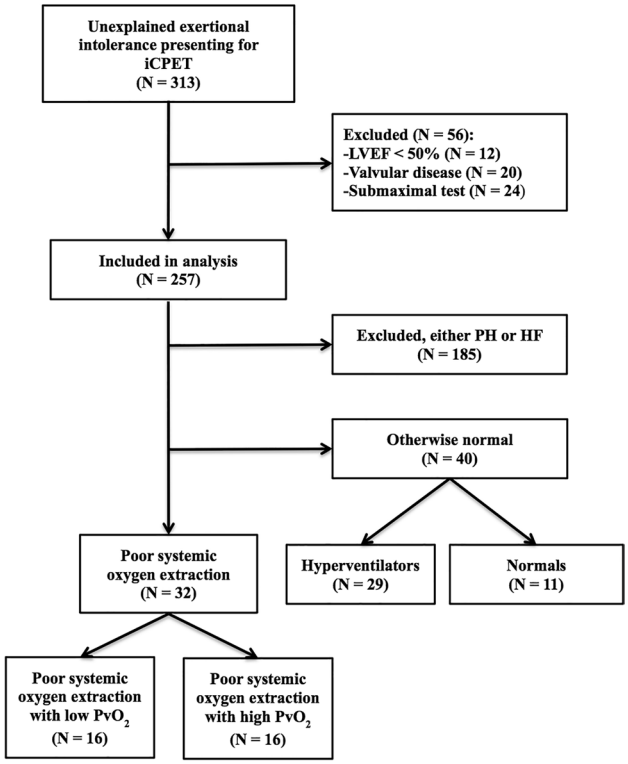

Fig. 1 Study population. *iCPET* invasive cardiopulmonary exercise test, *LVEF* left ventricular ejection fraction, *PH* pulmonary hypertension, *HF* heart failure, PvO_2 partial pressure of oxygen in mixed venous blood

Table 1 Poor SOE and normal SOE group inclusion criteria

Variables at peak exercise	Poor SOE	Normal SOE
$(Ca\text{-}vO_2)/[Hb]$	≤ 0.8	> 0.8
VO2	$< 80\%$ predicted	$\geq 80\%$ predicted
PVR[a]	< 120 dynes*sec/cm^5	< 120 dynes*sec/cm^5
mPAP[a]	< 30 mmHg	< 30 mmHg
Q_t[b]	$\geq 80\%$ predicted	$\geq 80\%$ predicted
PCWP[b]	< 20 mmHg	< 20 mmHg

[a]The PVR and mPAP were used to exclude those with pulmonary hypertension

[b]The Q_t and PCWP were used to exclude those with heart failure

cardiac echo (Fig. 1). Predicted heart rate was equal to 220 minus the patient's age. For purposes of this study, a normal exercise response was defined as $VO_{2max} \geq 80\%$ predicted $(Ca\text{-}vO_2)/[Hb] > 0.8$, and absence of pulmonary hypertension (PH), heart failure (HF), and pulmonary mechanical limit (PML) (Table 1). PML was defined as $V_{Emax}/MVV > 70\%$, where V_{Emax} is ventilation at peak exercise and MVV is maximum voluntary ventilation. Abnormal SOE was defined as $VO_{2max} < 80\%$ predicted $(Ca\text{-}vO_2)/[Hb] \leq 0.8$ (Wasserman et al. 2012), and the absence of PH, HF, and PML. PH was defined as peak exercise mPAP ≥ 30 mmHg and a peak exercise PVR ≥ 120 dynes*sec/cm^5 with concurrent peak exercise PCWP < 20 mmHg (Groves et al. 1987; Reeves et al. 1988; Tolle et al. 2008b). HF was defined as peak exercise $Q_t < 80\%$ predicted and peak exercise PCWP ≥ 20 mmHg (Santos et al. 2015).

$\Delta Q_t/\Delta VO_2$ was derived from the respective variables at the start of and at peak exercise. The relationship of V_E/VCO_2 versus VO_2 throughout exercise was derived using breath-by-breath data from rest to peak exercise, where V_E is minute ventilation (Arena et al. 2003; Guazzi et al. 2005).

Since poor extraction was associated with hyperventilation, we subdivided normals to determine if hyperventilation independently affects exercise performance. To do so, we defined hyperventilators as those whose peak exercise $PaCO_2$ was $< [1.5 \times HCO_3 + 6]$ as predicted by Winters' formula (Albert et al. 1967), which has been validated at peak incremental exercise (Wasserman et al. 2014). The otherwise normal group was then subdivided into hyperventilators (HV) and non-hyperventilators (Fig. 1). Those who were normal and did not hyperventilate were called normals (NL). All variables were compared across the three groups: poor SOE, HV, and NL. We next split the poor SOE group by median PvO_2 at peak exercise in an attempt to distinguish between true skeletal muscle mitochondrial dysfunction, which would elevate PvO_2 (poor SOE_H) (Taivassalo et al. 2002), and those with a low PvO_2 (poor SOE_L) and left shift of the oxyhemoglobin dissociation curve. The two subsets, poor SOE_L and poor SOE_H, were compared to NL and HV across all variables (Fig. 1).

Subgroup analyses were also conducted to address possible confounders for exercise ventilatory control and microcirculatory function. To control for the influence of sex hormones on respiratory control, we repeated our analysis after excluding males (NL group had an insufficient number of females remaining for statistical analysis), and to control for medication effects, we performed our analysis after excluding those taking vasoactive medications (beta blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers).

Statistical analysis

Continuous variables are expressed as mean ± standard deviation. Categorical variables are expressed as number of subjects and proportion, *n* (%). One-way analysis of variance with the Bonferroni correction was used to perform multiple group comparisons. Fisher's exact test was applied to compare proportions. A two-sided *p* value of < 0.05 was considered significant. Statistical analysis was performed using Stata software Version 12.1 (Stata Corp LP, College Station, TX, USA).

Results

Prevalence of poor systemic oxygen extraction

Of the 313 subjects referred for iCPET, 257 were available for analysis after the initial exclusion criteria were applied (Fig. 1). Of those excluded, 12 subjects had LVEF < 50%, 20 had significant valvular disease, and 24 performed a submaximal test. Of the remaining 257 eligible for analysis, 32 (12.5%) had poor systemic oxygen extraction (SOE) and 40 (15.6%) were considered otherwise normal. By study design, the poor SOE group was then split by median peak PvO_2, resulting in 16 (6.2%) in each SOE_L and SOE_H. Among the otherwise normal group, 29 (11.3%) were hyperventilators (HV) and 11 (4.3%) were normals (NL). The three groups' (NL, HV, poor SOE) demographics were similar with the exception of sex and age; there were the fewest females in NL and the most in poor SOE, while HV was older than the others. The groups also were similar with regards to baseline co-morbidities and medications taken at the time of testing, except that there was a difference in beta blocker usage among the three groups (Table 2). Additionally, it should be noted that among the poor SOE population, the SOE_L group was responsible for the majority (75%) of beta blocker usage and 100% of the ACE inhibitor, angiotensin II receptor blocker, calcium channel blocker, and diuretic use. Other clinical diagnoses for the poor SOE group are also described (Table 3).

Ventilation and oxygenation

At rest, the three groups had equivalent arterial and mixed venous pH (pHa and pHv), PaO_2, $PaCO_2$, $PvCO_2$, and SaO_2. HV had lower resting PvO_2 compared to poor SOE and lower resting SvO_2 compared to poor SOE and NL. Otherwise, poor SOE and NL groups had similar mean resting PvO_2 and SvO_2 (Table 4, Fig. 2).

At peak exercise, HV and poor SOE had less acidemic pHa and pHv compared to NL. This relative alkalemia was explained by relative hypocapnia in both arterial and mixed venous samples compared to NL. Poor SOE and HV had increased V_E/VCO_2 compared to NL (33.5 ± 8.1 vs. 31.5 ± 5.2 vs. 24.5 ± 3.0, $p < 0.001$) (Fig. 3). In other words, both poor SOE and HV had excessive minute ventilation necessary for the carbon dioxide output of the exercising muscle.

As a result of hyperventilation, at maximum exercise, HV and poor SOE had higher PaO_2 and SaO_2 compared to NL. In the mixed venous compartment, poor SOE had

Table 2 Demographic data

Characteristic or medication at time of iCPET study	NL ($N=11$)	HV ($N=29$)	Poor SOE ($N=32$)	P value
Age (years)	42 ± 13	58 ± 13	47 ± 17	0.002[a]
Female sex, N (%)	4 (36)	17 (59)	27 (84)	0.007[b]
Body mass index (kg/m^2)	24.9 ± 3.9	26.4 ± 4.5	25.8 ± 6.4	0.74
Hypertension, N (%)	1 (9)	11 (38)	8 (25)	0.17
Diabetes mellitus, N (%)	0 (0)	1 (4)	1 (3)	0.82
Obesity, N (%)	3 (27)	9 (32)	12 (38)	0.80
Coronary artery disease, N (%)	0 (0)	2 (7)	0 (0)	0.22
Smoking history, N (%)[c]	1 (9)	0 (0)	1 (3)	0.42
Atrial fibrillation, N (%)	0 (0)	3 (10)	0 (0)	0.10
Calcium channel blocker, N (%)	0 (0)	3 (10)	4 (9)	0.55
Beta blocker, N (%)	0 (0)	5 (17)	12 (38)	0.04[b]
ACE inhibitor or ARB, N (%)[d]	0 (0)	7 (24)	4 (13)	0.18
Diuretic, N (%)[e]	0 (0)	5 (17)	5 (16)	0.28
CoEnzyme Q, N (%)	0 (0)	1 (4)	2 (6)	0.65
Carnitine, N (%)	0 (0)	0 (0)	2 (6)	0.28
Creatine, N (%)	0 (0)	0 (0)	1 (3)	0.53
Magnesium, N (%)	1 (9)	3 (10)	2 (6)	0.84
Vitamin B6, N (%)	1 (9)	2 (7)	3 (9)	0.94

[a]HV differs from the others

[b]All three differ from each other

[c]Smoking history defined as current or prior smoker

[d]*ACE* angiotensin-converting enzyme, *ARB* angiotensin II receptor blocker

[e]Diuretic = a thiazide diuretic or a loop diuretic

Table 3 Clinical characteristics of poor SOE

Clinical diagnosis	Present
Mitochondrial myopathy[a]	22 (69%)
Dysautonomia[b]	18 (56%)
Connective tissue disease[c]	6 (19%)
None of the above	2 (6%)
Confirmatory diagnostic test	12 (38%)
Small fiber neuropathy	5 (16%)[d]
Tilt table test	4 (13%)[d]
Genetic mutation analysis	3 (10%)

[a]Mitochondrial myopathy (MM) includes clinically suspected MM, with or without confirmative genetic mutation analysis or muscle biopsy

[b]Dysautonomia includes clinically suspected postural orthostatic tachycardia syndrome (POTS), dysautonomia, and/or orthostatic hypotension, with or without confirmative tilt table test and skin biopsy

[c]Connective tissue disease (CTD) includes those with clinically diagnosed connective tissue disease and/or autoimmune antibody titers suggestive of CTD

[d]Three patients had positive tilt table testing and skin biopsy showing small fiber neuropathy, resulting in nine unique patients (29%) with confirmatory diagnoses of MM or dysautonomia

significantly higher PvO_2 and SvO_2 compared to HV and NL (Fig. 2). These findings remained significant after excluding males or those taking vasoactive medications (supplemental tables). When the poor SOE group was divided by median PvO_2, at peak exercise, SOE_L was associated with a greater respiratory alkalosis in both arterial and mixed venous compartments, i.e., a subset of poor SOE was characterized by hyperventilation, respiratory alkalemia, and a left shift of the oxyhemoglobin dissociation curve. While the arterial lactate in the SOE_L group was lower than the others', the serum bicarbonate was equivalent, suggesting that the relatively higher pH was largely driven by respiratory rather than metabolic acid–base changes. Conversely, the subset with higher PvO_2 also had an elevated SvO_2, consistent with a primary defect of oxygen uptake and utilization (Santos et al. 2015) (Table 4, Fig. 2). These changes were seen at equivalent metabolic rates (VO_{2max} percent predicted) and cardiac outputs (as a percent predicted).

Rest and exercise Fick principle variables and hemodynamics

At rest, poor SOE had lower VO_2 compared to NL. The groups also differed across Q_t, cardiac index (CI), Ca-vO_2, HR, SBP, PVR, and SVR. The remainder of the hemodynamic parameters at rest including [Hb], DBP, RAP, mPAP, and PCWP were similar among the groups (Table 5).

All subjects achieved maximal effort based on the RER > 1.0 and HR > 80% predicted at end exercise. At maximum exercise, compared to the other two groups, poor SOE had the lowest absolute VO_2, VO_{2max} percent predicted (which is adjusted for age, sex, and height), and Ca-vO_2, as dictated by the study design. HV had a lower absolute VO_2 at peak exercise compared to NL, and the percent predicted VO_{2max}, while still normal (per study design), trended lower than NL's. The peak workload was different among all three major groups (there was no difference between the poor SOE subgroups). Poor SOE also had lower mPAP and V_E and higher SVR when compared to the other two. This difference in peak SVR remained after excluding those taking vasoactive medications and dividing the poor SOE group into poor SOE_L and poor SOE_H. If fact, the poor SOE_L had the highest SVR among the four groups (Table 5). There was no difference in SVR between HV and poor SOE when males were excluded.

At maximum exercise, NL had higher absolute Q_t and CI than the other two groups, but Q_t percent predicted (adjusted for age, sex, and height) was not different. Lastly, the DBP was higher in poor SOE compared to HV. Absolute maximum HR, HR percent predicted, SBP, respiratory rate (RR), RER, RAP, PCWP, and PVR were not different among the three groups. Of note, peak HR and percent predicted maximal HR were significantly lower in poor SOE_L (Table 5).

Despite equivalent percent predicted Q_{tmax}, $\Delta Q_t/\Delta VO_2$ was higher for poor SOE compared to the others, HV and NL, respectively (8.2 ± 1.8 vs. 6.2 ± 1.0 vs. 5.3 ± 1.2, $p < 0.001$, Fig. 4).

Discussion

Exertional intolerance has many causes, and after an initial noninvasive work up, a subset of patients remains without a diagnosis. Invasive cardiopulmonary exercise testing has proven to be a useful diagnostic modality, elucidating the rate-limiting pathophysiology of exertional intolerance (Borlaug et al. 2010; Maron et al. 2013; Santos et al. 2015; Tolle et al. 2008b). In the current study, we identified a group of patients with poor systemic oxygen extraction as the sole identifiable cause of exertional intolerance.

Of the 257 patients who presented with unexplained exertional intolerance and met our criteria for analysis, 12.5% had evidence of poor systemic oxygen extraction as their only apparent abnormality. Poor SOE was defined as low (< 80% predicted) systemic oxygen uptake (VO_{2max}) and narrower than expected difference between arterial and venous oxygen content (Ca-vO_2) at maximum exercise in the absence of a cardiopulmonary limitation. At peak exercise, when most of the mixed venous blood is being returned from the exercising limb muscle, oxygen should be maximally extracted from the arterial blood resulting in a Ca-vO_2, whose numeric value is directly proportional to the

Table 4 Arterial and venous blood samples at rest and peak exercise

	Rest					Peak				
	NL ($N=11$)	HV ($N=29$)	Poor SOEL ($N=16$)	Poor SOEH ($N=16$)	*P* value	NL ($N=11$)	HV ($N=29$)	Poor SOEL ($N=16$)	Poor SOEH ($N=16$)	*P* value
Arterial										
pH	7.43 ± 0.03	7.44 ± 0.03	7.43 ± 0.03	7.43 ± 0.04	0.826	7.31 ± 0.02	7.38 ± 0.03	7.41 ± 0.05	7.37 ± 0.04	< 0.001[a–d]
PaO_2 (mmHg)	118 ± 19	107 ± 15	106 ± 16	112 ± 13	0.192	94 ± 16	110 ± 16	113 ± 16	119 ± 13	0.001[a–c]
$PaCO_2$ (mmHg)	34.1 ± 4.2	34.5 ± 5.5	32.6 ± 5.1	34.6 ± 4.7	0.646	37.5 ± 3.4	31.6 ± 5.4	29.3 ± 5.3	30.6 ± 5.2	0.001[a–c]
SaO_2 (% sat)	98.7 ± 0.46	98.2 ± 0.70	98.3 ± 0.73	98.4 ± 0.56	0.151	96.3 ± 2.4	97.9 ± 1.5	98.4 ± 0.7	98.3 ± 0.4	0.001[a–c]
Bicarbonate (mmol/L)	23.0 ± 1.9	24.1 ± 2.9	22.1 ± 3.3	23.7 ± 2.4	0.159	19.9 ± 2.0	19.3 ± 3.3	18.8 ± 2.5	19.5 ± 3.7	0.8347
Lactate (mg/dL)	0.8 ± 0.3	0.8 ± 0.2	0.9 ± 0.4	1.2 ± 1.4	0.348	6.5 ± 1.9	6.7 ± 2.1	4.7 ± 1.6	7.2 ± 3.3	0.016[d,e]
Venous										
pH	7.39 ± 0.02	7.40 ± 0.03	7.40 ± 0.03	7.40 ± 0.03	0.867	7.21 ± 0.04	7.25 ± 0.04	7.31 ± 0.06	7.26 ± 0.06	< 0.001[a–e]
PvO_2 (mmHg)	38 ± 2	35 ± 3	35 ± 3	40 ± 3	< 0.001[d,f]	26 ± 4	25 ± 3	28 ± 2	33 ± 1	< 0.001[c–f]
$PvCO_2$ (mmHg)	40.6 ± 3.2	39.8 ± 7.5	38.8 ± 5.0	39.4 ± 5.2	0.874	65.5 ± 8.5	55.9 ± 10.4	49.4 ± 10.1	52.1 ± 4.2	< 0.001[a–c]
SvO_2 (% sat)	70 ± 3	65 ± 6	67 ± 4	72 ± 4	< 0.001[a,d,f]	31.4 ± 7.0	31.4 ± 8.0	44.1 ± 5.2	48.6 ± 4.4	< 0.001[b,c,e,f]
Bicarbonate (mmol/L)	25.5 ± 2.0	25.0 ± 4.0	25.9 ± 6.7	24.9 ± 2.7	0.909	27.2 ± 2.4	25.9 ± 4.2	25.7 ± 3.5	24.3 ± 2.9	0.228

[a]NL differs from HV

[b]NL differs from poor SOE_L

[c]NL differs from poor SOE_H

[d]Poor SOE_L differs from poor SOE_H

[e]HV differs from poor SOE_L

[f]HV differs from poor SOE_H

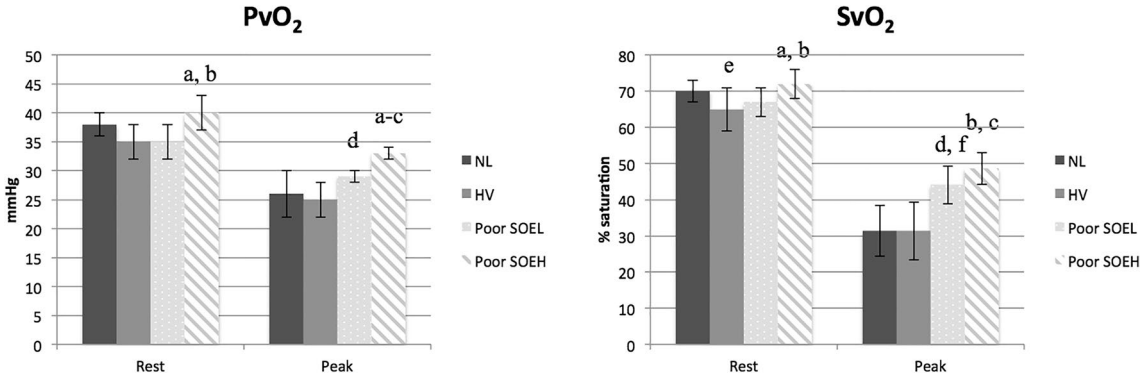

Fig. 2 Mixed venous oxygen partial pressure and hemoglobin saturation at rest and peak exercise. Comparison of mixed venous blood samples drawn at rest versus peak exercise. Poor systemic oxygen extraction (SOE) group with high PvO_2 (SOE_H) had a significant elevation in peak PvO_2. Both SOE_H and poor SOE with lower PvO_2 (SOE_L) had elevated SvO_2 at peak exercise, suggesting poor oxygen off-loading or uptake at the level of the systemic capillary. a = poor SOE_H differs from poor SOE_L ($p<0.05$); b = poor SOE_H differs from hyperventilators (HV) ($p<0.05$); c = poor SOE_H differs from normals (NL) ($p<0.05$); d = poor SOE_L differs from HV ($p<0.05$); e = HV differs from NL ($p<0.05$); f = poor SOE_L differs from NL ($p<0.05$)

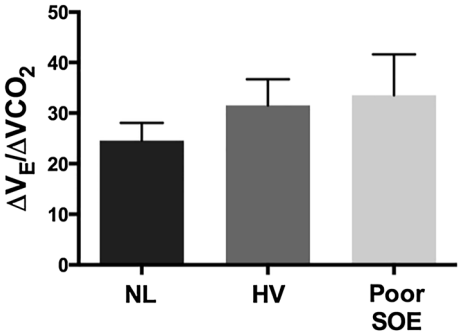

Fig. 3 Relationship of minute ventilation to change in carbon dioxide production during exercise in normals, hyperventilators, and those with poor systemic oxygen extraction. The ratio of change in the minute ventilation (V_E) compared to the change in carbon dioxide produced (VCO_2) from rest to maximum exertion is compared among the three groups and is significantly different ($p<0.001$). Poor systemic oxygen extraction (SOE) and hyperventilators (HV) had have excessive ventilation for the carbon dioxide produced compared to normals (NL) (33.5 ± 8.1 vs. 24.5 ± 3.0, $p=0.001$ and 31.5 ± 5.2 vs. 24.5 ± 3.0, $p=0.010$, respectively). There was no difference between HV and poor SOE ($p=0.740$). This is demonstrated by a larger relative increase in minute ventilation than was necessary for carbon dioxide produced by the skeletal muscle

hemoglobin concentration (Wasserman et al. 2012). However, in the current study, we found a group of symptomatic patients unable to fully extract the oxygen delivered to the exercising muscles, which in turn depresses their aerobic capacity, given that peak cardiac output was normal and unable to compensate further.

Our data further suggest that there are three mechanisms that contribute to impaired peripheral oxygen extraction: (1) primary dysfunction of the skeletal muscle mitochondrion, (2) hyperventilation and a left shift of the oxyhemoglobin dissociation curve, and (3) limb muscle microcirculatory dysregulation. These mechanisms are likely interrelated, each exacerbating the effect of the other with the end result of poor SOE (Fig. 5), as demonstrated by decreased VO_{2max} and Ca-vO_2.

Primary oxygen extraction defect

A principal finding of the current study was a high prevalence of poor SOE as the only cause of exertional symptoms and decreased aerobic capacity. At maximum exercise, the components of oxygen delivery were normal (Q_t percent predicted and [Hb]) or elevated (SaO_2), while SvO_2 at maximum exercise was higher than normal for poor SOE. Thus, by the Fick principle ($VO_{2max}=[Q_{tmax}]\times[Ca\text{-}vO_{2max}]$), poor oxygen extraction was implicated as the sole cause of decreased VO_{2max}. Impaired oxygen extraction as the primary cause of decreased VO_{2max} is the hallmark of MM. Healthy subjects rely on an intact mitochondrial respiratory chain to create an oxygen pressure gradient whereby oxygen off-loading from blood to tissue is favored. Without a "sump" for oxygen, normal oxygen extraction is not possible.

Similar to previous results from largely noninvasive exercise tests of patients with confirmed MM (Bravo et al. 2012; Flaherty et al. 2001; Gimenes et al. 2011; Heinicke et al. 2011; Lindholm et al. 2004; Taivassalo et al. 2002, 2003), using iCPET we found that a cohort of patients had poor SOE as the only identifiable cause of their exertional intolerance, as shown by a commensurate decrease of VO_{2max} and Ca-vO_2, increased cardiac output response to exercise, and increased ratio of V_E to VCO_2 over the course of exercise. In a teleological sense, it is likely that a hyperdynamic cardiopulmonary response to exercise is an adaptive response designed to augment oxygen delivery to muscle when its

Table 5 Cardiopulmonary variables at rest and peak exercise

Variable	Rest					Peak				
	NL ($N=11$)	HV ($N=29$)	Poor SOEL ($N=16$)	Poor SOEH ($N=16$)	*P* value	NL ($N=11$)	HV ($N=29$)	Poor SOEL ($N=16$)	Poor SOEH ($N=16$)	*P* value
VO_2 (L/min)	362 ± 111	319 ± 106	298 ± 63	255 ± 56	0.022[b]	2725 ± 800	1893 ± 657	1160 ± 283	1169 ± 169	< 0.001[a–c,e,f]
VO_{2max} (% predicted)						117 ± 47	102 ± 16	68 ± 10	70 ± 6	< 0.001[b,c,e,f]
Peak workload (W)						223 ± 76	147 ± 56	82 ± 20	106 ± 20	< 0.001[a–c,e,f]
Q_t (L/min)	6.7 ± 2.7	4.9 ± 1.7	5.1 ± 1.2	5.6 ± 1.9	0.044[a]	19.2 ± 4.8	14.4 ± 3.7	12.4 ± 2.5	12.8 ± 2.2	< 0.001[a–c]
Q_t (% predicted)						116 ± 42	111 ± 19	103 ± 14	107 ± 163	0.419
CI (L/min/m^2)	3.5 ± 1.5	2.4 ± 0.7	2.7 ± 0.6	3.3 ± 1.0	0.006[a,f]	9.8 ± 2.0	7.4 ± 1.8	6.5 ± 1.0	7.9 ± 1.4	< 0.001[a–c]
Ca-vO_2 (mL/100 mL)	5.6 ± 1.0	6.7 ± 1.4	6.0 ± 1.1	4.8 ± 1.0	< 0.001[d,f]	14.2 ± 2.5	13.0 ± 1.9	9.4 ± 1.1	9.2 ± 1.2	< 0.001[b,c,e,f]
[Hb] (g/dL)	14.6 ± 1.3	13.8 ± 1.8	13.6 ± 1.1	13.3 ± 1.1	0.157					
RER						1.20 ± 0.1	1.17 ± 0.1	1.15 ± 0.1	1.31 ± 0.2	0.001d,f
RR (min^{-1})						34 ± 6	35 ± 8	38 ± 17	32 ± 11	0.598
V_E (L/min)						85 ± 30	75 ± 27	53 ± 14	50 ± 12	< 0.001[b,c,e,f]
HR (min^{-1})	68 ± 12	72 ± 14	77 ± 8	86 ± 17	0.003[c,f]	148 ± 28	141 ± 24	130 ± 25	159 ± 24	0.012[d]
HR (% of predicted)[g]						83 ± 16	87 ± 12	77 ± 9	90 ± 12	0.029[d]
SBP (mmHg)	136 ± 19	146 ± 17	150 ± 25	132 ± 18	0.036	185 ± 37	168 ± 35	177 ± 36	170 ± 18	0.470
DBP (mmHg)	76 ± 12	79 ± 11	81 ± 13	73 ± 9	0.168	81 ± 16	77 ± 18	88 ± 15	87 ± 8	0.042
RAP (mmHg)	2 ± 2	3 ± 3	4 ± 3	2 ± 2	0.167	7 ± 4	7 ± 4	7 ± 4	4 ± 4	0.339
Mean PAP (mmHg)	12 ± 2	13 ± 3	15 ± 2	12 ± 2	0.023[d]	27 ± 8	26 ± 5	23 ± 4	22 ± 4	0.014
PCWP (mmHg)	7 ± 3	6 ± 3	7 ± 3	4 ± 2	0.010[d]	13 ± 2	13 ± 4	12 ± 3	11 ± 5	0.344
PVR (dynes*sec/cm^5)	76 ± 30	126 ± 40	118 ± 47	123 ± 47	0.010[a,c]	58 ± 31	72 ± 26	68 ± 38	74 ± 24	0.492
SVR (dynes*sec/cm^5)	1323 ± 494	1841 ± 622	1706 ± 410	1463 ± 512	0.027	500 ± 117	636 ± 221	795 ± 244	735 ± 130	0.001[b]
FEV1/FVC[g]	77 ± 9	78 ± 7	83 ± 7	84 ± 4	0.002[b,c]					
V_{Emax}/MVV						35 ± 26	33 ± 22	37 ± 17	40	0.976

[a]NL differs from HV

[b]NL differs from poor SOE_L

[c]NL differs from poor SOE_H

[d]Poor SOE_L differs from poor SOE_H

[e]HV differs from poor SOE_L

[f]HV differs from poor SOE_H

[g]*FEV1/FVC* forced expiratory volume over 1 s divided by forced viral capacity

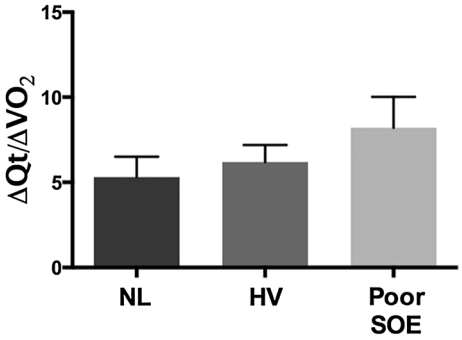

Fig. 4 Cardiac output response to exercise in normals, hyperventilators, and those with poor systemic oxygen extraction. The ratio of change in the cardiac output (Q_t) compared to the change in oxygen consumption (VO_2) from rest to maximum exertion is compared among the three groups and is significantly different ($p<0.001$). Patients with impaired systemic oxygen extraction (SOE) had an exaggerated cardiac output response to exercise, compared to normals (NL) (8.2 ± 1.8 vs. 5.3 ± 1.2, $p<0.001$) and hyperventilators (HV) (8.2 ± 1.8 vs. 6.2 ± 1.0, $p<0.001$). There was no difference between NL and HV ($p=0.157$). This is demonstrated by a larger relative increase in cardiac output than was necessary for the oxygen utilized by the exercising peripheral skeletal muscle

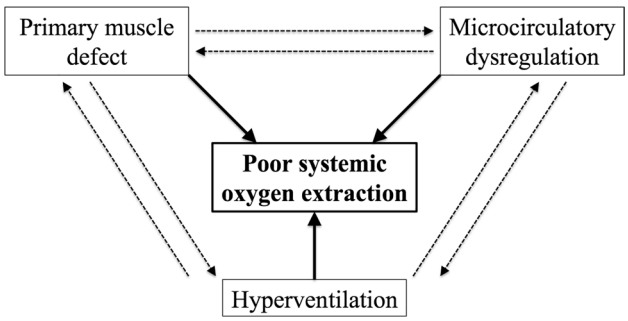

Fig. 5 Three mechanisms influence poor systemic oxygen extraction. We describe a cohort of patients with dyspnea whose sole identifiable cause of their symptoms is poor systemic oxygen extraction (SOE). Poor SOE is caused by three interrelated mechanisms: a primary skeletal muscle mitochondrial defect, limb muscle microcirculatory dysregulation, and hyperventilation. Intrinsic mitochondrial dysfunction impedes oxygen off-loading. Microcirculatory changes, perhaps reflected by an increased SVR, contribute to a limb muscle perfusion-metabolism mismatch, decreasing oxygen delivery to the mitochondrion. Hyperventilation blunts adaptive acidemia and the Bohr effect, as well as a blood flow "steal" phenomenon away from the exercising muscle. These mechanisms compound one another and exacerbate poor oxygen off-loading in the systemic capillary, causing poor systemic oxygen extraction and resultant dyspnea on exertion

peripheral uptake and utilization are impaired. These data support the hypothesis that a subset of patients with "unexplained" exertional intolerance has dysfunction at the level of skeletal muscle resulting in limited oxygen extraction, in the absence of heart failure, pulmonary hypertension, or primary pulmonary mechanical limitation.

The nature of our database is a clinical referral system whereby many of the patients came from outside of BWH, and as a result, systematic follow-up of their clinical course was not available for all cases. All of our cohort with poor SOE carried a clinical diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome (Bested and Marshall 2015). We were additionally able to identify three general disease categories: MM, dysautonomia, and connective tissue disease. Although about two-thirds were referred with suspected MM, most did not hold a confirmed diagnosis by muscle biopsy or blood mutation screens at the time of iCPET. MM is a difficult diagnosis to make, and many patients with MM have negative DNA mutation screens in blood and muscle biopsies (Taivassalo et al. 2003; Tarnopolski 2004), as was the case here. Concordant increases in peak PvO_2 and SvO_2 observed in a subset of our poor SOE are typical of primary mitochondrial defects in skeletal muscle (Taivassalo et al. 2002). Our results suggest a subset of patients with unexplained exercise intolerance share an iCPET phenotype similar to those with a well-defined skeletal muscle mitochondrial mutation. Moving forward, as we accumulate a larger database of patients, we will be able to subdivide and describe this group further with numbers large enough to perform statistical analysis among subgroups. Currently, we have a descriptive analysis of patients who share a common cause or phenotype of their exertional intolerance.

Hyperventilation and the oxyhemoglobin dissociation curve

Functional mitochondria and the Bohr effect (whereby oxygen off-loading is favored in acidic or hypercapnic conditions) are required for normal systemic oxygen extraction at maximum exercise (Stringer et al. 1994; Wasserman et al. 2012). This process is driven by the acid pH in the systemic capillary blood compartment, which decreases the affinity of hemoglobin for oxygen, resulting in a right shift of the oxyhemoglobin dissociation curve (Stringer et al. 1994; Wasserman et al. 2012).

In the current study, poor SOE and HV hyperventilated (the latter by study design). This resulted in both less acidemic arterial and mixed venous blood at peak exercise compared to NL, from which one can infer that the systemic capillary was similarly affected. Since the bicarbonate and lactate concentrations at peak exercise were equivalent among the groups, this blunted acidemia is attributed exclusively to relative hypocapnia. Furthermore, prior to the SOE group split, equivalent lactate concentrations suggest that each major group had similar byproducts of glycolysis, and the poor SOE group did not simply produce less carbon dioxide because of a decreased effort. That said, it should be noted that when the SOE group was split, the poor SOE_H

group did have an increased lactate production compared to the SOE_L group. We speculate that this elevation in lactate in SOE_H may reflect this group's mitochondrial dysfunction rather than less effort. Further, predicted PvO_2 at peak exercise is 27 mmHg (Hansen et al. 1984), which was seen only in the NL group. The other three groups had significantly lower PvO_2, and, therefore, the SOE_L group's relative alkalemia was driven by respiratory rather than metabolic changes. It is of interest that HV, although defined by normal aerobic capacity and systemic oxygen extraction, tended to show impairments in both when compared to normals.

Poor SOE and HV do not have an appropriate drop in systemic capillary pH at maximum exercise and lose the normal physiologic rightward shift of the oxyhemoglobin dissociation curve. Compared to healthy individuals with the same PvO_2, these patients will not off-load as much oxygen to the tissues and have a resultant elevation in SvO_2. Our results are similar to those of Hayashi et al. (1999), who showed that hyperventilation causes a left shift of the oxyhemoglobin dissociation curve during exercise.

It is reasonable to ask why our HV group did not have a statistically significant impairment of systemic oxygen extraction. This is because our study design first dictated normal oxygen extraction in this group. Nonetheless, we identified a trend toward impaired systemic oxygen extraction in HV, which was intermediate between that of NL and poor SOE_L. We posit that this hyperventilation leads to less adaptive acidemia, which is necessary during exercise to promote oxygen off-loading in the tissues (Stringer et al. 1994).

Poor SOE and HV had excessive minute ventilation relative to carbon dioxide output, suggested by an increased V_E/VCO_2 relationship measured throughout incremental exercise. A noninvasive CPET may, therefore, serve as a useful screen for this exercise phenotype.

When we split by median PvO_2 at maximum exercise, two poor oxygen extractor phenotypes are suggested: those with primary extraction defects at the level of the skeletal muscle and those with impaired oxygen off-loading from hemoglobin from a left shift. As demonstrated by Taivassalo and colleagues (2003), we reasoned that those with higher peak PvO_2 were more likely to have mitochondrial disease, as evidenced by elevated PvO_2 suggesting no "sump" for oxygen diffusion. We speculate higher arterial lactate was consistent with more impaired oxidative metabolism in this group.

Conversely, poor SOE with a lower PvO_2 had more hypocapnic and less acidemic mixed venous blood suggesting a similar finding in the muscle capillary. Both groups had equal degrees of poor extraction (decreased VO_{2max} and decreased Ca-vO_2). We do not believe lower HR and arterial lactate at peak exercise suggested a submaximum effort in SOE_L, since Q_{tmax} percent predicted was the same and the PvO_2 nadir was no different from normal. Likewise, a lower RER peak in SOE_L is expected, because submaximum exercise hyperventilation increases carbon dioxide-buffering capacity in blood. In sum, we suggest poor SOE can be caused by impaired oxygen off-loading associated with a primary defect of skeletal muscle mitochondrial function, hyperventilation, or a combination of both.

Hyperpnea without hyperventilation may also decrease VO_{2max} regardless of pH and $PvCO_2$. Chin and colleagues have recently suggested that isocapnic hyperpnea results in a sympathetically mediated blood flow "steal" phenomenon, diverting cardiac output away from the locomotor and towards the respiratory muscles (Chin et al. 2013; Hayashi et al. 1999). Impaired limb skeletal muscle blood flow then causes decreased VO_{2max} in this patient population that has reached maximal HR and Q_t, as dictated by study design. In addition, the slow twitch muscle fibers of the normal human diaphragm are served by arteriolar resistors less susceptible to sympathetic vasoconstriction than those of the limb (Aaker and Laughlin 2002). As such, diversion of cardiac output toward the diaphragm could be expected to decrease maximum work and VO_{2max}, albeit without additional impairment of peripheral oxygen extraction (Aaron et al. 1992). Taken together, our data suggest that hyperventilation decreases VO_{2max} most significantly by impairing oxygen off-loading in the peripheral tissues, and also possibly through redistribution of blood flow away from the exercising limb skeletal muscle.

The mechanism of pathologic hyperventilation among those with poor SOE or MM is not known, although the muscle metaboreflex has been implicated in physiologic hyperventilation (Oelberg et al. 1998). Feedback systems via Group IV afferent nerves from skeletal muscle metaboreceptors to respiratory and cardiovascular control centers (Amann et al. 2010; Eldridge 1994; Evans et al. 1998; Kaufman 2010; Kaufman and Hayes 2002; Oelberg et al. 1998; Vissing et al. 2001; Williamson 2010) may be activated in MM (Heinicke et al. 2011). Others have shown a link between hyperventilation and decreased or slowed blood flow to the exercising muscle as well (Chin et al. 2010). More work needs to be done to fully explain the hyperventilatory state consistently seen in patients with MM, or more broadly, poor SOE.

Microcirculatory dysfunction

Another possible reason for poor SOE is skeletal muscle microcirculatory structural change or microcirculatory dysfunction, both of which have been described in the literature in other disease processes. The latter has been found in disorders such as neuropathic POTS and other forms of dysautonomia, which represent a large percentage of clinical diagnoses in our patient population. Such patients have

a dropout of distal autonomic neurons (Mar and Raj 2014), which results in blunted arterial vasoconstriction and venous pooling (Stewart 2002). It seems biologically plausible that inadequate arteriolar resistor function in the limb skeletal muscle would lead to de facto left-to-right shunting and impaired systemic oxygen extraction in such patients.

Increased muscle sympathetic nerve activity, linked to a local metaboreflex, has been observed in heart failure and POTS and likely contributes to a maladaptive increase in SVR (Piepoli and Crisafulli 2014). Our poor SOE_L subgroup had the highest SVR among all four subgroups, supporting the theory that there is an aberrant neuroendocrine response to exercise in a subset of patients leading to elevated SVR in addition to hyperventilation. Although we do not have direct data on perfusion/metabolism heterogeneity within the exercising muscle bed, our finding of increased SVR associated with poor SOE suggests the possibility that blunted sympatholysis contributes to impaired systemic oxygen extraction (Greaney et al. 2013).

The use of vasoactive medications does not affect this microcirculatory change, as indicated by our subgroup analysis before the SOE group was split. Additionally, beta blocker usage is not thought to affect peripheral oxygen extraction. Additionally, when males were excluded from the analysis, there was no difference seen in peak SVR seen between HV and poor SOE. Although not previously documented, estrogen levels may play a role in these microcirculatory changes. Future work should be done to investigate the role of sex hormones in microcirculatory regulation.

Summary

Impaired systemic oxygen extraction is an important pathophysiologic mechanism underlying unexplained exertional intolerance. Taken together, the root causes of impaired SOE, in turn include hyperventilation, skeletal muscle mitochondrial defect, and limb muscle microcirculatory dysfunction, all of which likely interact (Fig. 5).

Limitations

Our normal group may not have been truly "normal," as all subjects presented with symptomatic exertional intolerance. Although those designated as "normal" do not have any identifiable pathology based on the available hemodynamic and hematologic parameters set for this study, they may still perform differently than healthy controls. Recruitment of a truly normal control population for iCPET is likely unethical. However, this consideration does not discount our findings. In fact, in comparison to a truly healthy cohort, our findings may become even more exaggerated.

In theory, a primary supranormal cardiac output could lead to a subsequent decreased "need" to extract oxygen during exercise. However, it is difficult to envision that this group had supranormal cardiac output because by definition they had VO_{2max} percent predicted in the abnormal range; i.e., they were not well-trained athletes and their maximum cardiac output was equal to, but not greater than, predicted. Thus, it is more plausible that the primary insult is poor extraction, with a compensatory increase in the ratio of Q_t/VO_2.

The disproportionate use of vasoactive mediations, particularly beta blockers, in the poor SOE_L group may have confounded some of the physiologic findings here. There is a lower maximum percent predicted heart rate in this group compared to the SOE_H group, and medications could certainly be a culprit. However, since the maximum percent predicted Q_t was equivalent among all groups, these patients compensated for their chronotropic insufficiency by augmenting stroke volume. Calcium channel blockers usage among SOE_L, by causing arteriolar and venous dilatation, may have theoretically created left to right shunting. However, this physiologic change would mimic the MM phenotype in the SOE_H group, rather than the hyperventilatory phenotype that was seen in the SOE_L group. Furthermore, our subgroup analysis excluding those taking vasoactive medications showed no difference in our results, indicating that these medications were not the cause of poor systemic oxygen extraction.

It is expected that patients with exertional intolerance lead a more sedentary life than a true normal population. Therefore, could deconditioning explain many of the findings of poor SOE? While deconditioning can lower Ca-vO_2 slightly, it should only decrease Ca-vO_2 by approximately 1 mL/dL (Carrick-Ranson et al. 2014). The average difference observed here between poor SOE and NL was 4 mL/dL, suggesting deconditioning is not the sole reason for poor extraction.

There are three methods available to calculate the V_E/VCO_2 relationship during exercise, which can be referred to as "ventilatory efficiency" in some cases: measuring the V_E/VCO_2 slope up to the respiratory compensation point, the V_E/VCO_2 fraction at the ventilatory anaerobic threshold, or the change over the entire CPET as we have done. Prior work suggests that this latter method is superior to the former two methods in predicting outcomes (Arena et al. 2003; Guazzi et al. 2005). Furthermore, in our specific subgroup of patients who hyperventilate at submaximal exercise, we are not able to reliably detect either the ventilatory anaerobic threshold or the respiratory compensation point due to the burden of hyperventilation during submaximum exercise. In our study, the V_E/VCO_2 relationship was not our major focus, since we directly measured hyperventilation via radial artery blood gas and

pH analysis. However, for centers investigating undifferentiated dyspnea with noninvasive CPET, it is important to note ventilatory inefficiency is a nonspecific finding.

Our blood gases were not temperature corrected. However, exercise-induced temperature rises were unlikely to play a major role in our observed blood gas differences. Some authors do not believe that blood gas interpretation should be temperature corrected, since the oxygen and carbon dioxide content (different than partial pressure) does not change with temperature shifts (Hansen 1989). Others have shown that the temperature rise in arterial and mixed venous blood during short-term incremental cycling is small, up to 1 °C from rest to peak (Brudin 1975; Calbet and Boushel 2015). This temperature change causes small change in pH and the partial pressures of carbon dioxide and oxygen. If muscle temperature rose more in NL than HV and poor SOE as a result of more exercise, the temperature-corrected $PaCO_2$ and $PvCO_2$ in both compartments would have been higher than our reported values uncorrected at 37 °C; this would have actually magnified the differences between NL and the other three groups with respect to hyperventilation. With regard to Ca-vO_2 differences, any temperature rise would have similarly affected the small contributions of dissolved oxygen in the arterial and mixed venous compartments, with no net change in our principal findings.

Our mixed venous blood gas samples did not allow for a direct measurement of muscle intracellular or capillary blood gases and pH. Near-infrared spectroscopy and ^{31}P-magnetic resonance spectroscopy show that there can be changes intracellularly that are not reflected by venous blood samples (Argov et al. 1987; Arnold et al. 1985; Bravo et al. 2012; Taylor et al. 1994; Trenell et al. 2006). We and others have previously shown that the exercising component of muscle becomes more acidotic than the blood compartment (Evans et al. 1998; Kowalchuk et al. 1988; Oelberg et al. 1998), because intracellular pH changes are mitigated by the buffering capacity of systemic blood. However, while acid production in the exercising muscle contributes to local capillary pH, it is local the capillary, not intracellular, pH that affects the oxyhemoglobin dissociation curve. Since we know the pH in the arterial and mixed venous compartments, we can assume that the capillary pH lies somewhere in between these values, even if affected by the pH of surrounding muscle. Therefore, our data give us insight into the local capillary pH and its effects on oxygen off-loading to the exercising muscle. It may be possible that after the remaining blood volume joins that returning from the exercising leg, torso, heart, and diaphragm muscles, the mixed venous pH is more alkalotic than the local pH in the capillary. If this was the case and we had measured local pH, then we would anticipate our findings would be more exaggerated than they are with this mixed venous blood assessment.

Femoral venous sampling might be a more direct method of estimating muscle capillary blood gases and pH, and such an approach would complement the current study. Maximum incremental exercise, however, has the advantages of determining the relative contributions of the Fick principle variables to reduced VO_{2max}, ruling out confounding cardiac and pulmonary disease and allowing an estimated prevalence of poor SOE as the sole reason for impaired aerobic capacity. Finally, it is possible or even likely that whole body (as opposed to isolated muscle group) exercise is necessary to elicit the full maladaptive neuroendocrine response, including hyperventilation and sympathetic activation, which is relevant to our poor SOE phenotype. A more focused study on the exercising muscle, such as a one-leg knee extensor protocol, is an attractive future study.

Conclusion

Using direct measurements of the Fick principle variables, we have identified a cohort of patients whose exertional intolerance is caused solely by poor systemic oxygen extraction. These patients demonstrate a hyperventilatory and hypercirculatory pattern, which may be driven by an exaggerated muscle metaboreflex. While for some, there appears to be a primary defect in skeletal muscle mitochondrial oxygen uptake (PvO_2 at maximum exercise is high), for others, the predominant pathophysiology appears to be hyperventilation that causes a left shift of the oxyhemoglobin dissociation curve and impaired oxygen off-loading in the systemic capillary.

Impaired systemic oxygen extraction should be added to the differential diagnosis of unexplained exertional intolerance. In addition, exercise hyperventilation should be recognized for its deleterious effects on oxygen transport, rather than being dismissed as inconsequential or psychosomatic.

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Author contributions KM, MS, RO, MU, AO, AW, and DS performed data collection and analysis. DF and DM performed study design. KM and DS wrote the manuscript. All the authors reviewed, edited, and approved the manuscript.

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Compliance with ethical standards

Conflict of interest ABW and DMS funded by NIH 2R01HL060234-12A1 and U01HL125215-01. ARO supported by the Dunlevie Family Fund. The remaining authors have no conflicts of interest.

Ethical approval All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964

Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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