Northumbria Research Link

Northumbria
University
NEWCASTLE

Respiratory and locomotor muscle blood flow during exercise in health and chronic obstructive pulmonary disease

Ioannis Vogiatzis[1], Zafeiris Louvaris[2], PD Wagner[3]

1. Department of Sport, Exercise and Rehabilitation, Northumbria University Newcastle, Newcastle, UK
2. Department of Rehabilitation Sciences, Faculty of Kinesiology and Rehabilitation Sciences, Rehabilitation for Internal Disorders Research Group, KU Leuven, Leuven, Belgium
3. Division of Pulmonary, Critical Care, and Sleep Medicine, Department of Medicine, University of California San Diego, San Diego, CA, USA.

New findings

Using an approach we developed to measure muscle blood flow by indocyanine green dye detected by near infrared spectroscopy, we have found that isocapnic hyperpnoea at rest leads to an increase in respiratory muscle blood flow that is proportional to the respiratory muscle work required. Surprisingly, cycling exercise interferes with respiratory muscle blood flow, especially in COPD, but even in health athletes. Intercostal muscle blood flow during exercise fails to reach flow rates observed at the same minute ventilation as during isocapnic hyperpnoea, and in COPD, intercostal muscle blood flow during exercise actually falls below flow during resting breathing. No evidence is found in intact subjects for redistribution of blood flow from the legs to the respiratory muscles during heavy exercise in health or COPD. Evidence of decrease in leg blood flow and increase in respiratory muscle flow was found only when imposing expiratory flow limitation (EFL) during exercise in healthy individuals. However, because EFL caused substantial physiological derangement, these results cannot be projected onto normal exercise.

This is an Accepted Article that has been peer-reviewed and approved for publication in the Experimental Physiology, but has yet to undergo copy-editing and proof correction. Please cite this article as an Accepted Article; doi: 10.1113/EP088104.

Abstract

We have developed an indicator-dilution method to measure muscle blood flow at rest and during exercise using the light absorbing tracer indocyanine green dye (ICG) injected as an intravenous bolus, with surface optodes placed over muscles of interest to record the ICG signal by near-infrared spectroscopy. Here we review findings for both quadriceps and intercostal muscle blood flow (measured simultaneously) in trained cyclists and in patients with COPD. During resting hyperpnoea in both athletes and patients, intercostal muscle blood flow increased with ventilation, correlating closely and linearly with the work of breathing, with no change in quadriceps flow. During graded exercise in athletes, intercostal flow at first increased, but then began to fall approaching peak effort. Unexpectedly, in COPD, intercostal muscle blood flow during exercise fell progressively from resting values, contrasting sharply with the response to resting hyperpnoea. During exercise at peak intensity, we found no quadriceps blood flow reduction in favour of the respiratory muscles in either athletes or patients. In COPD at peak exercise, when patients breathed 21% oxygen in helium or 100% oxygen, there was no redistribution of blood flow observed between legs and respiratory muscles in either direction. Evidence of decrease in leg blood flow and increase in respiratory muscle flow was found only when imposing expiratory flow limitation (EFL) during exercise in healthy individuals. However, because EFL caused substantial physiological derangement, lowering arterial oxygen saturation and raising end-tidal PCO_2 and heart rate, these results cannot be projected onto normal exercise.

Main document

The work presented here focuses mostly on testing the theory of blood flow redistribution from the locomotor to the respiratory muscles during heavy exercise (Dempsey et al., 2006). This theory is based on evidence that during maximal exercise there is a decrease in blood flow to the locomotor muscles when respiratory muscle work is artificially increased and cardiac output (CO) is maximal (Harms et al., 1997;1998). By inference, the fraction of CO not directed to the leg muscles was presumed to be redirected to the respiratory muscles to support the high work of breathing (Dempsey et al., 2006). Along this line of thinking, unloading the respiratory muscles via non-invasive ventilation techniques was found to increase blood flow to the exercising locomotor muscles, inferring redistribution of flow away from the respiratory muscles to support the legs and allow greater exercise (Harms et al., 1997). However, in each of these studies, due to absent technology, respiratory muscle blood flow could not be measured to directly determine whether it did in fact increase when locomotor perfusion was noted to be reduced or fall when the respiratory muscles were unloaded, so the conclusions remained speculative.

We have partially addressed this methodological gap by applying the well-known indicator-dilution method that was initially validated by Boushel et al. 2000 for the locomotor muscles and later for the respiratory muscles by our group (Guenette et al., 2008) in a novel manner. We used the light absorbing tracer indocyanine green dye (ICG) given by intravenous bolus, with signals recorded by near-infrared spectroscopy (NIRS) from optodes taped to the skin over the left seventh intercostal space at the apposition of the costal diaphragm, and also over the vastus lateralis muscle. We measure the accumulation of ICG in the respiratory muscles using NIRS, while the concentration of ICG in the arterial blood is measured using photodensitometry (Boushel et al., 2000; Guenette et al., 2008). This technique has the advantage of being able to quantify absolute muscle blood flow (in ml/min/100gr) while providing simultaneous measurement of cardiac output (Boushel et al., 2000; Guenette et al., 2008).. For understanding circulatory regulation of respiratory muscles during exercise in healthy individuals and patients with COPD we assessed the perfusion of respiratory muscles over the intercostal muscles because these muscles as compared to other respiratory muscles (i.e. scalene and sternocleidmastoid) provide the main pressures to displace the ribcage and increase end-inspiratory lung volume whilst the diaphragm acts primarily as a flow generator (Aliverti et al., 1997). We also acknowledge that the selection

of intercostal muscles has potential limitations including that NIRS penetration depth over the seventh intercostal space reflects both internal and external intercostal muscles whilst NIRS measures at this site do not allow partitioning of blood flow between the intercostal muscles and the diaphragm (Guennete et al., 2008; Vogiatzis et al., 2009). Furthermore, it has been suggested that at high lung volumes NIRS measurements at intercostal muscles might capture perfusion of lung tissue and/or movement-related artefacts could also affect the blood flow measurements (Sheel et al., 2018). A difficulty in interpreting and comparing the results of our study with others is twofold. First, no other studies in this topic have measured the perfusion of intercostal muscles during exercise in healthy people or patients and secondly a very limited number of studies have simultaneously assessed blood flow responses in respiratory and limb muscles during exercise.

Data were first obtained in healthy subjects at rest during voluntary, isocapnic step increases in minute ventilation (V_E) from resting V_E to peak exercise V_E. This work showed a linear relationship (r^2=0.94, $p<0.01$) between blood flow and the work of breathing over the whole range of V_E, thereby establishing the method (Vogiatzis et al., 2009). Similar findings were noted in chronic obstructive pulmonary disease (COPD) patients during isocapnic hyperpnoea between blood flow and the power of breathing from resting V_E to peak exercise V_E (r^2=0.92, $p<0.01$, Vogiatzis et al., 2010).

We now describe a series of studies in healthy subjects and in patients with COPD in which respiratory and locomotor muscle blood flow were assessed at rest and during exercise. In one such study, in 8 healthy young subjects we compared vastus lateralis and intercostal muscle blood flow index at constant load exercise under two conditions: control, and with expiratory flow limitation (EFL) via a Starling resistor limiting expiratory flow to ~1 l/s (Athanasopoulos et al., 2010). Indeed, if arterial blood cannot be sampled, measuring just the rate of increase of ICG under the NIRS optode gives a quantitative parameter we call blood flow index (BFI) which has been shown to be proportional to blood flow (Habazzetl et al., 2010; Louvaris et al., 2018). This index can be compared across optode sites and experimental conditions to infer relative blood flow changes. Exercise work rate was set at peak EFL exercise work rate that was determined during an incremental exercise test with Starling resistor in place (120W), and duration was set at 6 minutes. CO increased to about 15 L/min and was slightly higher with EFL at any time point, but it was still clearly submaximal under both conditions. EFL perturbed the system greatly (V_E: 35 l/min vs 55 l/min control; SaO_2 89% vs 98%; end-tidal PCO_2 56 mm Hg vs 44 mm Hg). In every subject, EFL raised

intercostal muscle BFI and reduced quadriceps muscle BFI, ie, there was evidence of "steal" of blood flow from leg to respiratory muscles (Figure 1). The rise in respiratory muscle blood flow with EFL was expected because of the much greater work of breathing, but what was unexpected was that leg blood flow fell, because CO was far from maximal and therefore the cardiovascular system should have easily been able to sustain leg blood flow at the control level (Harms et al., 1998). Recently Dominelli et al. (2017) and Katayama et al. (2015; 2019) investigated the effect of increasing respiratory muscle work (via resistors) on sympathetic vasoconstrictor outflow and limb muscle blood flow in healthy individuals during submaximal and maximal exercise. Authors' results confirm ours as compared to exercise without respiratory resistance, sternocleidomastoid muscle blood flow was increased (Dominelli et al., 2017) whilst sympathetic vasoconstrictor outflow and limb muscle blood flow were reduced (Dominelli et al., 2017 and Katayama et al., 2015; 2019). Nevertheless, we suggest that the substantially perturbed physiology during EFL in our study, especially the hypercapnia and hypoxemia, may have resulted in a generalized sympathetic discharge, causing vasoconstriction and explaining leg flow reduction as opposed to "steal" at these submaximal CO values. In any event, one cannot project these results onto normal (maximal) exercise because of the substantially perturbed physiology.

The question becomes whether during maximal exercise without manipulating respiratory muscle work there is redistribution of blood flow from the leg to the respiratory muscles at/near peak exercise to support the high work of breathing. Accordingly, we designed experiments using graded cycling exercise to maximal levels simultaneously assessing both respiratory and leg muscle blood flow by ICG-NIRS. In 10 athletes we measured intercostal and quadriceps muscle blood flow during: a) progressive isocapnic hyperpnoea at rest and b) graded (discontinuous) cycling exercise including several exercise bouts with intensities up to the limit of tolerance and fixed duration (Vogiatzis et al., 2009). The design involved reaching the same high levels of V_E in a) as noted spontaneously in b). The resting hyperpnoea results confirmed our original findings (Guenette et al., 2008), with a large, smooth, exponential increase in intercostal muscle blood flow from resting V_E to peak exercise V_E that correlated linearly with the work of breathing (Vogiatzis et al., 2009) (Figure 2). The exercise results were quite unexpected and in contrast to other studies that supported (directly or indirectly) the concept of redistribution of blood flow from the locomotor to the respiratory muscles in healthy individuals (Sheel et al., 2018): First, from rest up to about 60% of peak V_E, intercostal muscle blood flow was higher at any V_E compared to isocapnic

hyperpnoea (Figure 2). Second, intercostal blood flow then fell back to resting values with further increases in exercise, and then even to below blood flow at resting V_E over the upper 20% of the exercise range. Quadriceps muscle blood flow rose as expected up to about 80% of peak watts, but then also started to fall (modestly) at higher work rate (Figures 2 and 3). CO also rose as expected from rest to 75% of peak exercise, but then essentially plateaued over the upper 20% of exercise (Figure 3). Certainly, respiratory muscle flow did not increase at the expense of leg flow, or vice versa. Why did both quadriceps and intercostal blood flow both fall when CO was maintained, and where did the leg/respiratory muscle blood flow go to? Overall systemic vascular conductance was not falling, so the reduction in both leg and respiratory muscle flows at high work rates may reflect redistribution of blood flow away from any contracting muscle beds to other parts of the body. Specifically, mechanical impediment to both quadriceps and intercostal muscle perfusion due to intense muscle contraction and development of high intramuscular pressures might in turn decrease microvascular perfusion (Laughlin et al., 1996). However, the impact of reactive hyperaemia during the relaxation phase of the contraction cycle to compensate for the reduction in blood perfusion during the intense muscle contraction is an issue that could have been tested if the NIRS technology available had a sufficiently high time resolution. In addition, the reduction in intercostal blood flow could also be due to redistribution of blood flow within the respiratory muscles secondary to changes in muscle recruitment during heavy exercise The latter mechanism is supported by a recent study from (Dominelli et al., 2017) showing a further increase (and not decrease) in the sternocleidomastoid muscle perfusion when respiratory muscle work increased (via resistors) in healthy subjects during intense cycling exercise (90% peak work rate).

Understanding the concept of blood flow redistribution between respiratory and locomotor muscles is particularly pertinent to patients with lung disease who exhibit an increased work of breathing at rest and during exercise. Therefore, we subsequently focused our studies on patients with COPD and addressed the question of whether blood flow would be redistributed from the locomotor to respiratory muscles during intense exercise in a setting – COPD - where work of breathing is high. To be noted that the only evidence that has been published so far in clinical conditions comes from animal models with heart failure supporting a preferential distribution of blood flow from locomotor to respiratory muscles during exercise (McConnell & Lomax, 2006; Smith et al., 2017).

Our experimental approach was to investigate whether at the same breathing power, intercostal muscle blood flow during intense exercise was greater than during resting hyperpnoea, because in the latter case, respiratory and locomotor muscles do not have to compete for the available blood flow since CO is clearly submaximal. We reasoned that if at the same power of breathing, intercostal muscle blood flow during intense exercise was greater than during resting hyperpnoea and quadriceps muscle blood flow was simultaneously decreased, this would provide evidence that there was an increase in blood flow in favour of the intercostal muscles – that is, a "steal".

We measured quadriceps and intercostal muscle blood flow and CO during resting hyperpnoea and from rest to peak exercise in 5 steps in 10 COPD patients (FEV_1: 51% predicted) (Vogiatzis et al., 2010). Hyperpnoea results again mirrored those of Guenette et al (2008), with an exponential rise in intercostal muscle blood flow from rest to peak isocapnic ventilation. Exercise results showed CO increasing until about 75% peak work rate and then plateauing. Quadriceps muscle blood flow increased linearly with the increase in work rate. Unexpectedly, intercostal muscle blood flow *fell* progressively from rest to peak exercise (Figure 4). In fact, intercostal muscle flow began to fall even during light exercise, and as CO was rising. As CO plateaued, a greater fall in intercostal flow now occurred. There was clearly no evidence of "steal" from legs to respiratory muscles; if anything one could argue the opposite from the dramatic reduction in intercostal flow. However, with intercostal muscle blood flow falling even as CO was increasing, it did not seem that a limited CO was the cause of intercostal muscle blood flow reduction. Again, one wonders if the greater work of breathing in COPD resulted in a greater contraction-induced mechanical obstruction to intercostal muscle blood flow that was more evident than in healthy subjects. Redistribution of blood flow within the respiratory muscles might also have been possible as expiratory abdominal muscle recruitment was more evident during exercise than isocapnic hyperpnoea (pressure time product during expiration was greater during exercise compared to isocapnic hyperpnoea and was linearly increased with the increase in work rate during cycling) (Vogiatzis et al., 2010).

The second experiment in COPD was designed to resemble the experimental approach adopted by Harms and colleagues (Harms et al., 1997;1998) albeit using a different method of unloading the respiratory muscles during exercise (i.e., heliox vs proportional assisted ventilation (PAV) adopted by Harms and colleagues). Of course, heliox does not unload respiratory muscles during exercise to the same extent as PAV. Thus, greater unloading with

PAV may explain, at least in part, the discrepancy between the current findings and those of Harms et al. (1997; 1998). Specifically we investigated whether at the same leg power, intercostal muscle blood flow during exercise with reduced breathing power induced by breathing 21% oxygen in helium (heliox) is lower and leg blood flow is higher compared to exercise in room air. (In COPD we cannot ethically further increase the work of breathing but we can reduce it). Here the reasoning was that if at the same leg power, intercostal muscle blood flow during heliox breathing was lower compared to air breathing, whilst quadriceps muscle blood flow was increased, this would have been evidence that there was a redistribution of blood flow from the intercostal to leg muscles (Vogiatzis et al., 2011). We measured CO, quadriceps and intercostal muscle blood flow during constant load exercise at three levels - 75%, 100% and 115% of peak work rate - during air and heliox in the same 10 COPD subjects (FEV_1: 51% predicted) as in previous work (Vogiatzis et al., 2010). Both quadriceps and intercostal muscle blood flow were a little higher (not lower) with heliox than air at 75% effort, but identical comparing air and heliox at 100% and 115% peak effort. CO was slightly higher with heliox at both work rates. Thus, reducing the work of breathing did not change relative quadriceps or intercostal muscle flow, and there was no redistribution between legs and intercostal muscles in either direction (Vogiatzis et al., 2011).

During subsequent constant-load (75% peak watts) exercise to the limit of tolerance in 10 COPD patients (FEV_1: 46% predicted) we compared breathing air, pure oxygen and heliox. We again assessed limb locomotor muscles and both intercostal and expiratory abdominal muscle blood flow and CO (Louvaris et al., 2014). Results showed similar, expected and substantial increases in endurance time with both heliox and oxygen compared to air breathing. We did not see any reduction in respiratory muscle (intercostal and expiratory abdominal muscle) blood flow with heliox or oxygen, in fact we found an increase in intercostal muscle blood flow with heliox. We also found an increase in quadriceps muscle blood flow with heliox but not with oxygen; neither heliox nor oxygen had an effect on CO. We reasoned that lack of reduction in respiratory muscle blood flow with heliox or oxygen rules out leg steal from respiratory muscles as explaining greater endurance, and that greater endurance was attributed to increased systemic and respiratory/locomotor muscle oxygen delivery (Louvaris et al., 2014).

Summary and considerations

Collectively, our studies and the direct experimental approach we adopted show that exercise interferes with respiratory muscle blood flow, especially in COPD, but even in health. During exercise, intercostal muscle blood flow fails to reach flows seen at the same V_E during isocapnic hyperpnoea; flow even falls to below resting values during heavy exercise. This dramatic, unanticipated flow reduction is much more evident in COPD than health, and requires explanation: It is seen even when cardiac output is submaximal, and may be due to mechanical obstruction to muscle blood flow from contractile compression of vessels within the muscle and/or due to redistribution of blood flow within the respiratory muscles. Importantly, in contrast to studies in healthy and animal models (Sheel et al., 2018) we found no evidence of blood flow steal from the legs in normal subjects or in COPD patients to support respiratory muscles during heavy exercise, nor any redistribution of blood flow between legs and respiratory muscles in either direction breathing air, heliox or 100% oxygen, with one exception - in normal subjects exposed to expiratory flow limitation severe enough to almost double end-tidal PCO_2 and cause arterial oxygen desaturation, where respiratory muscle flow increased while locomotor muscle blood flow decreased.

We conclude that due to technical limitations on assessing the perfusion of the diaphragm, an important step ahead for future studies aiming at directly addressing the theory of blood flow redistribution between locomotor and respiratory muscles requires the measurement of perfusion in more than a single respiratory muscle during exercise. NIRS-ICG derived BFI, representing a minimally invasive method, might allow future studies to focus on primary and secondary respiratory (scalene, parasternal, sternocleidomastoid, and abdominals) muscle perfusion responses and patterns, thus providing a more comprehensive resolution on this intriguing topic.

Authors Contribution

All authors have been involved in the process of writing and revising the paper and have approved the final submission.

Figure 1: Intercostal and quadriceps muscle blood flow index: individual changes are shown from rest (i.e.: quiet breathing) to the end of exercise with and without expiratory flow limitation (EFL) in healthy young subjects (Athanasopoulos et al., 2010).

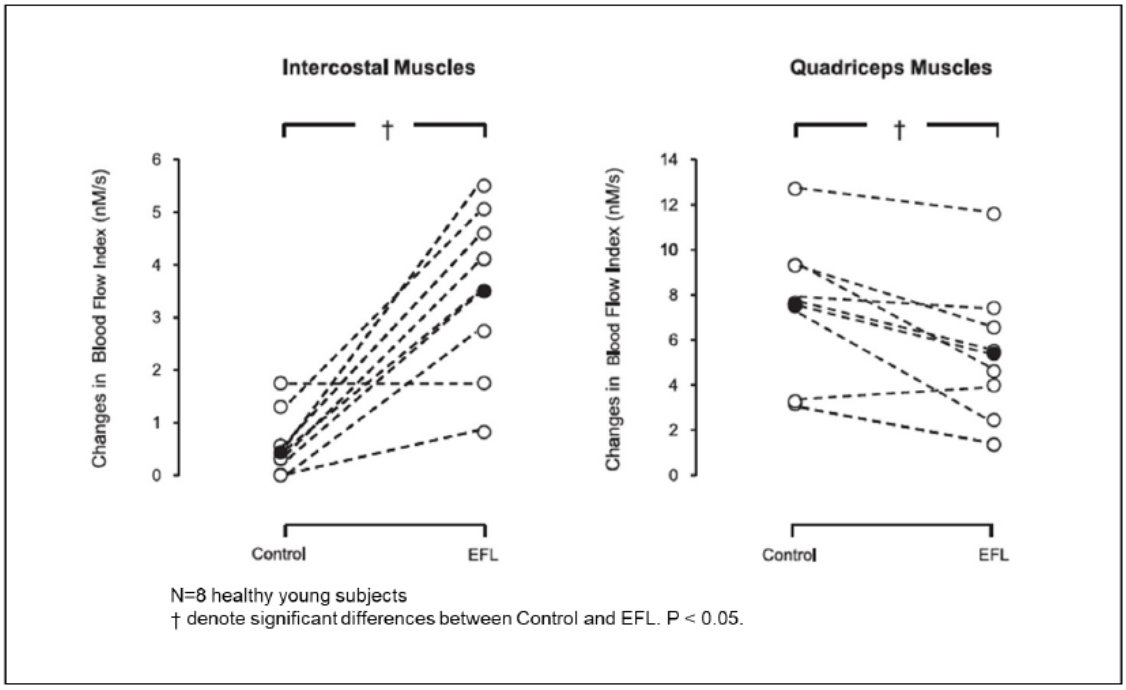

Figure 2: Quadriceps and intercostal muscle blood flow during graded (discontinuous) exercise (open symbols) and isocapnic resting hyperpnoea (closed symbols) for the intercostal (left panel) and quadriceps (right panel) muscles in athletes (Vogiatzis et al., 2009).

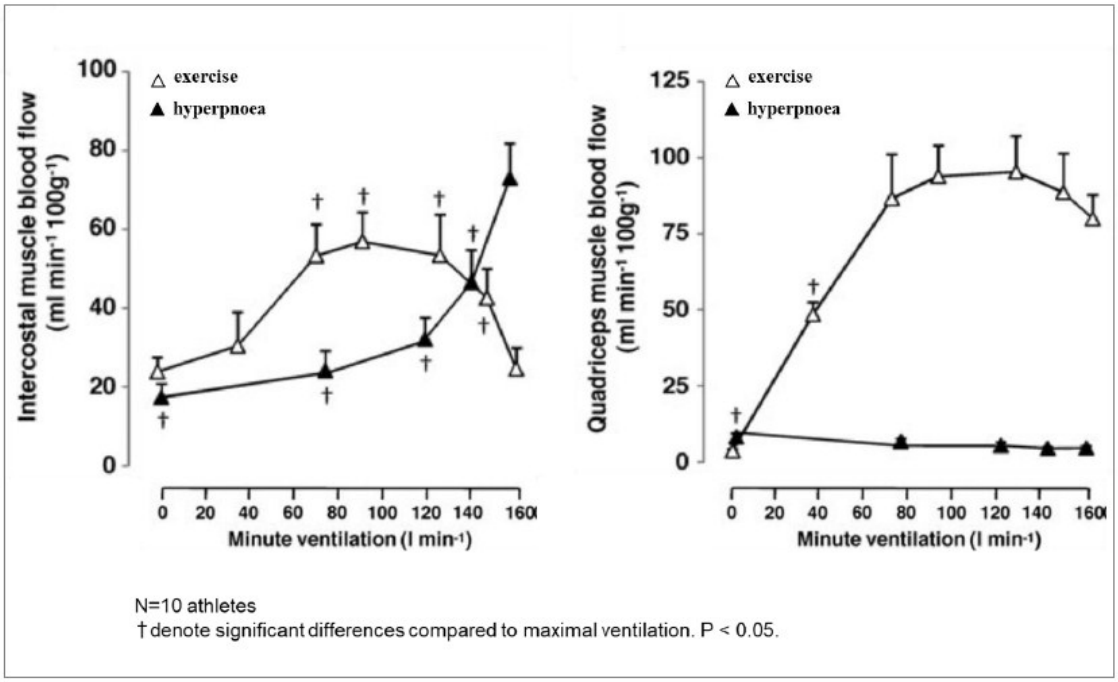

Figure 3: Left panel: Quadriceps and intercostal muscle blood flow, right panel: cardiac output, during graded (discontinuous) exercise in athletes (Vogiatzis et al., 2009).

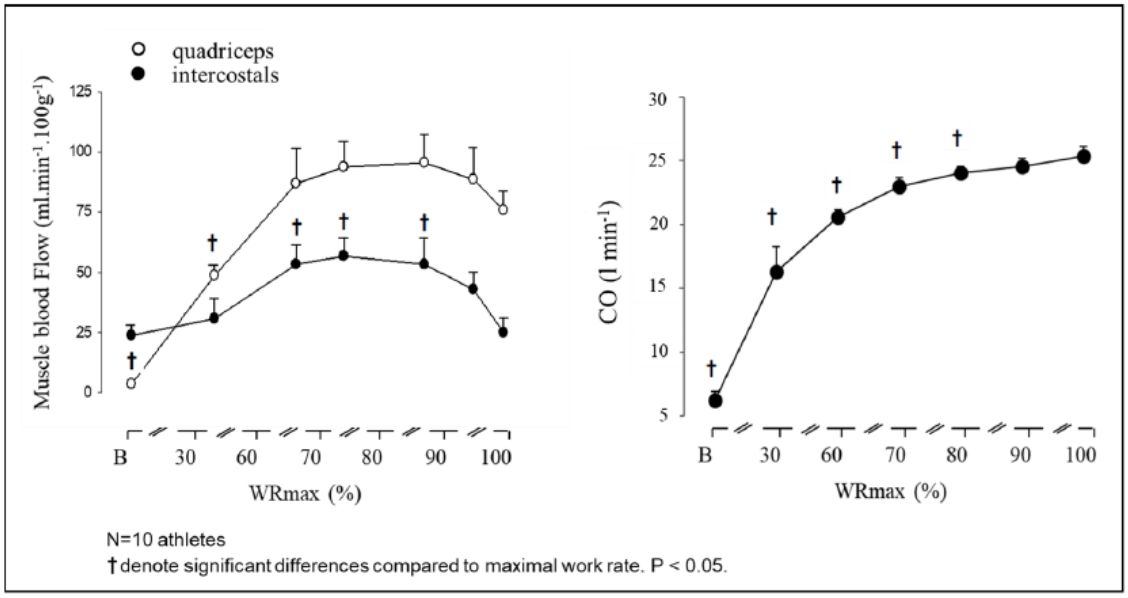

Figure 4: Left panel: intercostal muscle blood flow, right panel: quadriceps muscle blood flow during graded (discontinuous) exercise and isocapnic hyperpnoea reproducing exercise minute ventilation in patients with COPD. Mean minute ventilations corresponding rest and each exercise intensity are also presented (Vogiatzis et al., 2010).

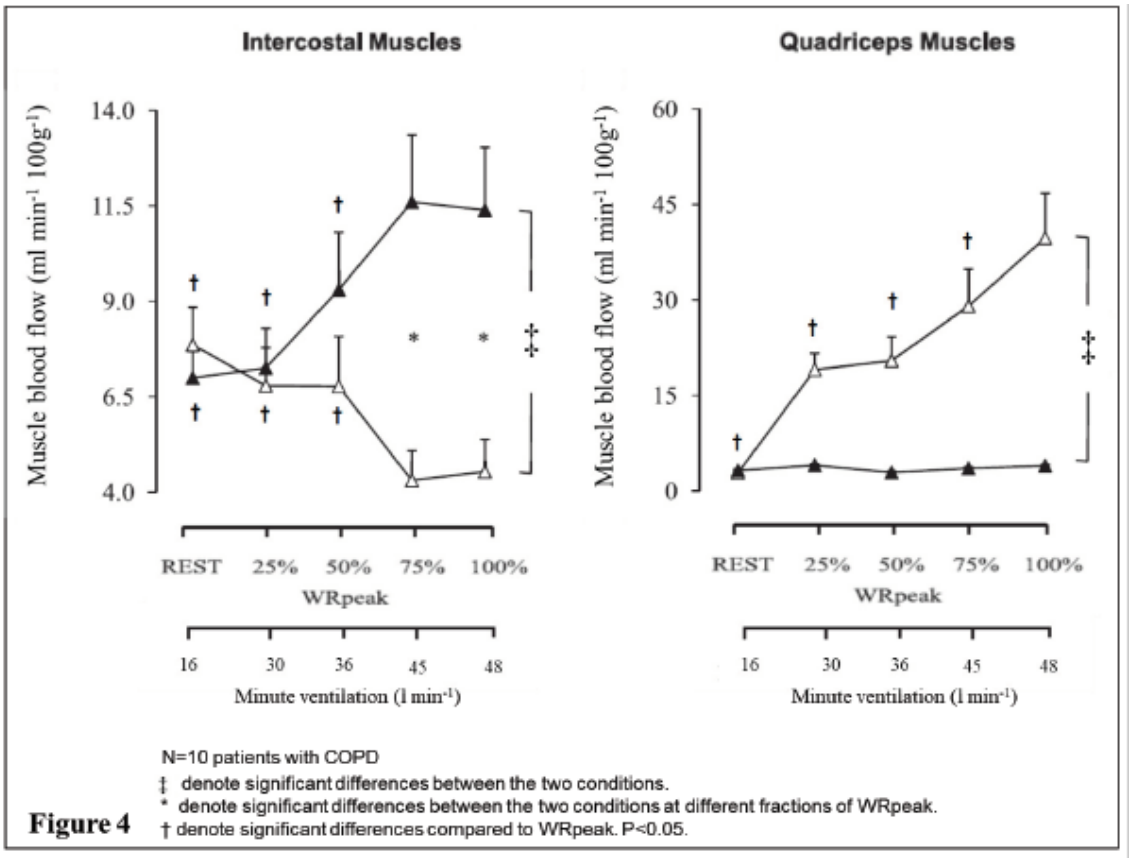

Figure 4

References

Aliverti, A., Cala, S.J., Duranti, R., Ferrigno, G., Kenyon, C.M., Pedoti, A.,…Yan, S. (1997). Human respiratory muscle actins and control during exercise. *Journal of Applied Physiology (1985)*, *83*(4), 1256–1269. https://doi.org/10.1152/jappl.1997.83.4.1256

Athanasopoulos, D., Louvaris, Z., Cherouveim, E., Andrianopoulos, V., Roussos, C., Zakynthinos, S., Vogiatzis, I. (2010). Expiratory muscle loading increases intercostal muscle blood flow during leg exercise in healthy humans. *Journal of Applied Physiology (1985), 109*(2), 388-395. https://doi.org/10.1152/japplphysiol.01290.2009

Boushel, R., Langberg, H., Olesen, J., Nowak, M., Simonsen, L., Bülow, J., Kjaer, M. (2000). Regional blood flow during exercise in humans measured by near-infrared spectroscopy and indocyanine green. *Journal of Applied Physiology (1985), 89*(5), 1868-78.

Dempsey, J.A., Romer, L., Rodman, J., Miller, J., Smith, C. (2006). Consequences of exercise-induced respiratory muscle work. *Respiratory Physiology and Neurobiology, 151*(2-3), 242-250. https://doi.org/10.1016/j.resp.2005.12.015

Dempsey, J.A., Sheel, A.W., St Croix, C.M., Morgan, B.J. (2002). Respiratory influences on sympathetic vasomotor outflow in humans. *Respiratory Physiology and Neurobiology, 130*(1), 3-20. https://doi.org/10.1016/S0034-5687(01)00327-9

Dominelli, P.B., Archiza, B., Ramsook, A.H, Mitchell, R.A., Peters, C.M., Molgat-Seon, Y.,…R., Sheel, A.W. (2017). Effects of respiratory muscle work on respiratory and locomotor blood flow during exercise. *Experimental Physiology 102*(11), 1535–1547. https://doi.org/10.1113/EP086566

Guenette J.A., Vogiatzis, I., Zakynthinos, S., Athanasopoulos D., Koskolou, M., Golemati, S., … Boushel, R. (2008). Human respiratory muscle blood flow measured by near-infrared spectroscopy and indocyanine green. *Journal of Applied Physiology (1985), 104*(4), 1202-10. https://doi.org/10.1152/japplphysiol.01160.2007

Habazettl, H., Athanasopoulos, D., Kuebler, W.M., Wagner, H., Roussos, C., Wagner P.D., …Vogiatzis, I. (2010). Near-infrared spectroscopy and indocyanine green derived blood flow index for noninvasive measurement of muscle perfusion during exercise. *Journal of Applied Physiology (1985), 108*(4), 962-967. https://doi.org/10.1152/japplphysiol.01269.2009

Harms, C.A, Babcock, M.A, McClaran, S.R., Pegelow, D.F., Nickele, G.A., Nelson, W.B., Dempsey, J.A. (1997). Respiratory muscle work compromises leg blood flow during maximal exercise. *Journal of Applied Physiology (1985), 82*(5), 1573-1583. https://doi.org/10.1152/jappl.1997.82.5.1573

Harms, C.A., Wetter, T.J., McClaran S.R., Pegelow, D.F., Nickele, G.A., Nelson, W.B., Hanson, P., Dempsey, J.A. (1998). Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. *Journal of Applied Physiology (1985), 85*(2), 609-618. https://doi.org/10.1152/jappl.1998.85.2.609

Katayama, K., Goto K., Shimizu, K., Saito, M., Ishida, K., Zhang, L.,… Sheel, AW. (2019). Effect of increased inspiratory muscle work on blood flow to inactive and active limbs during submaximal dynamic exercise. *Experimental Physiology 104*(2):180-188. https://doi.org/10.1113/EP087380

Katayama, K., Itoh, Y., Saito, M., Koike, T., Ishida, K. (2015). Sympathetic vasomotor outflow and blood pressure increase during exercise with expiratory resistance. *Physiological Reports 3*(5), e12421. https://doi.org/10.14814/phy2.12421

Laughlin, M.H., Korthuis, R.J., Duncker, D.J., Bache, R.J. (1996). Control of blood flow to cardiac and skeletal muscle during exercise. In Handbook of Physiology, section 12, Exercise: Regulation and Integration of Multiple Systems, ed. Rowell LB & Shepherd JT, pp. 705–769. American Physiological Society, Bethesda, MD, USA.

Louvaris. Z., Vogiatzis, I., Aliverti, A., Habazettl, H., Wagner, H., Wagner, P., Zakynthinos, S. (2014). Blood flow does not redistribute from respiratory to leg muscles during exercise breathing heliox or oxygen in COPD. Journal of Applied Physiology *(1985), 117*(3), 267-76. https://doi.org/10.1152/japplphysiol.00490.2014

Louvaris. Z., Habazettl, H., Wagner, H., Zakynthinos, S., Wagner, P.D., Vogiatzis, I. (2018). Near-infrared Spectroscopy Using Indocyanine Green Dye for Minimally Invasive Measurement of Respiratory and Leg Muscle Blood Flow in Patients With COPD. *Journal of Applied Physiology (1985), 125*(3), 947-959. https://doi.org/10.1152/japplphysiol.00959.2017

McConnell, A.K., Lomax, M. The influence of inspiratory muscle work history and specific inspiratory muscle training upon human limb muscle fatigue. (2006). *The Journal of Physiology, 577*(1): 445–457. https://doi.org/10.1113/jphysiol.2006.117614

Sheel, A.W., Boushel, R., Dempsey, J.A. (2018). Competition for blood flow distribution between respiratory and locomotor muscles: implications for muscle fatigue. *Journal of Applied Physiology (1985),125*(3):820-831.
https://doi.org/10.1152/japplphysiol.00189.2018

Smith, J.R., Hageman, K.S., Harms, C.A., Poole, D.C., Musch, T.I. Effect of chronic heart failure in older rats on respiratory muscle and hindlimb blood flow during submaximal exercise (2017). *Respiratory Physiology and Neurobiology, 243*, 20–26. https://doi.org/10.1016/j.resp.2017.05.002

Vogiatzis. I., Athanasopoulos, D., Habazettl, H., Aliverti, A., Louvaris, Z., Cherouveim, E., … Zakynthinos, S. (2010). Intercostal muscle blood flow limitation during exercise in chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine, 182*(9), 1105-1113. https://doi.org/10.1164/rccm.201002-0172OC

Vogiatzis, I., Athanasopoulos, D., Habazettl, H., Kuebler, W.M., Wagner, H., Roussos, C., … Zakynthinos S. (2009). Intercostal muscle blood flow limitation in athletes during maximal exercise. *Journal of Physiology, 587*(14), 3665–3677 https://doi.org/10.1113/jphysiol.2009.171694

Vogiatzis, I., Habazettl, H., Aliverti, A., Athanasopoulos, D., Louvaris, Z., LoMauro, A., … Zakynthinos S. (2011). Effect of helium breathing on intercostal and quadriceps muscle blood flow during exercise in COPD patients. *American Journal of Physiology Regulatory Integrative Comparative Physiology, 300*(6), R1549-59. https://doi.org/10.1152/ajpregu.00671.2010