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The Spatial Distribution Of Absolute Skeletal Muscle Deoxygenation During Ramp-Incremental Exercise Is Not Influenced By Hypoxia

T Scott Bowen[1], Shunsaku Koga[2], Tatsuro Amano[3], Narihiko Kondo[3], and Harry B Rossiter[4,5]

[1]Leipzig University - Heart Center, Department of Internal Medicine and Cardiology, Leipzig, Germany; [2]Applied Physiology Laboratory, Kobe Design University, Kobe, Japan; [3]Laboratory for Applied Human Physiology, Graduate School of Human Development and Environment, Kobe University, Kobe, Japan; [4]Rehabilitation Clinical Trials Center, Division of Respiratory & Critical Care Physiology & Medicine, Los Angeles Biomedical Research Institute and Harbor-UCLA Medical Center, Torrance, CA, USA; [5]School of Biomedical Sciences, University of Leeds, Leeds, UK.

Abstract Time-resolved near-infrared spectroscopy (TRS-NIRS) allows absolute quantitation of deoxygenated ([HHb]) haemoglobin and myoglobin concentration in skeletal muscle. We recently showed that the spatial distribution of peak [HHb] within the quadriceps during moderate-intensity cycling is reduced with progressive hypoxia and this is associated with impaired aerobic energy provision. We therefore aimed to determine whether reduced spatial distribution of skeletal muscle [HHb] was associated with impaired aerobic energy transfer during exhaustive ramp-incremental exercise in hypoxia. Seven healthy men performed ramp-incremental cycle exercise (20 W/min) to exhaustion at 3 fractional inspired O_2 concentrations (F_IO_2): 0.21, 0.16, 0.12. Pulmonary O_2 uptake ($\dot{V}O_2$) was measured using a flow meter and gas analyser system. Lactate threshold (LT) was estimated non-invasively. Absolute muscle deoxygenation was quantified by multichannel TRS-NIRS from the rectus femoris and vastus lateralis (proximal and distal regions). $\dot{V}O_{2peak}$ and LT were progressively reduced ($p<0.05$) with hypoxia. There was a significant effect ($p<0.05$) of F_IO_2 on [HHb] at baseline, LT, and peak. However the spatial variance of [HHb] was not different between F_IO_2 conditions. Peak total Hb ([Hb_{tot}]) was significantly reduced between F_IO_2 conditions ($p<0.001$). There was no association between reductions in the spatial distribution of skeletal muscle [HHb] and indices of aerobic energy transfer during ramp-incremental exercise in hypoxia. In conclusion, while regional [HHb] quantified by TRS-NIRS at exhaustion was greater in hypoxia, the spatial distribution of [HHb] was unaffected. Interestingly, peak [Hb_{tot}] was reduced at the tolerable limit in hypoxia implying a vasodilatory reserve may exist in conditions with reduced F_IO_2.

1 Introduction

In skeletal muscle during exercise the balance between the rate of oxygen utilisation ($\dot{V}O_2$) and oxygen delivery ($\dot{Q}O_2$) underlies the ability to meet cellular energetic demands through oxidative metabolism [1]. Heterogeneity in muscle fibre oxidative capacity, capillarity, blood flow, and recruitment means that skeletal muscle $\dot{Q}O_2/\dot{V}O_2$ is also widely heterogeneous within and between skeletal muscles [1]. A wide spatial heterogeneity in $\dot{Q}O_2/\dot{V}O_2$ may reflect a beneficial metabolic 'flexibility', i.e. by maintaining muscle regions with high PO_2 and thus high potential for oxidative energy provision and fatigue resistance. Alternatively, wide spatial heterogeneity in $\dot{Q}O_2/\dot{V}O_2$ may reflect a detrimental condition impairing oxidative energy provision in muscle regions that contribute to limiting the system's entire output (and thus limiting exercise tolerance). Whether spatial heterogeneity in $\dot{Q}O_2/\dot{V}O_2$ is a beneficial or detrimental physiological response to exercise remains unclear.

The dynamics of the $\dot{Q}O_2/\dot{V}O_2$ ratio is the predominant variable determining changes in the near-infrared spectroscopy (NIRS) derived deoxyhaemoglobin and myoglobin signal (hereafter termed HHb, for simplicity) during exercise. In humans, a hypoxia-induced slowing of $\dot{V}O_2$ kinetics during moderate intensity exercise is associated with reduced [HHb] heterogeneity [2]. This suggests that a narrow spatial distribution in $\dot{Q}O_2/\dot{V}O_2$ may reflect conditions detrimental to high rates of aerobic energy transfer. While the mechanisms matching regional $\dot{Q}O_2$ to $\dot{V}O_2$ remain equivocal, hypoxia may reduce nitric oxide (NO) bioavailability (a potent vasodilator) and limit the ability to maintain muscle regions with a high $\dot{Q}O_2/\dot{V}O_2$ ratio [3].

It remains unknown whether [HHb] heterogeneity is reduced during maximal exercise, where it has the potential to contribute to the mechanisms limiting exercise tolerance [1]. We therefore determined the association between the spatial distribution of skeletal muscle [HHb] and indices of aerobic energy transfer (pulmonary $\dot{V}O_{2peak}$ and lactate threshold) during ramp-incremental exercise to the limit of tolerance in normoxia and hypoxia. Absolute [HHb] was measured by multi-channel time resolved (TRS)-NIRS. We hypothesized that in hypoxia: (i) altered control of regional microvascular blood flow would cause the spatial distribution of [HHb] to become more uniform; and (ii) a reduced metabolic flexibility reflected by low [HHb] heterogeneity would correlate with reduced aerobic energy provision and exercise intolerance.

2 Methods

Seven healthy men (mean±SD: age, 22±2 years; height, 172±6 cm; weight, 61±6 kg; and $\dot{V}O_{2peak}$, 50±8 ml/kg/min) provided written informed consent, as approved by the Human Subjects Committee of Kobe Design University. Detailed descriptions of all procedures, equipment, and measurements have been previously published [2]. Briefly, a cycle ergometer ramp-incremental (RI) exercise test (20 W/min) was performed at 60 rpm to exhaustion with three inspired fractional oxygen concentrations (F_IO_2): 0.21; 0.16; and 0.12. Each test

was performed on a different day in a randomised order. Participants breathed through a mouthpiece connected to a low-resistance, two-way non-rebreathing valve (2700, Hans Rudolph, Shawnee, KS, USA), linked to rubber tubing that supplied humidified air from Douglas bags filled with room air (F_IO_2 = 0.21) or room air diluted with N_2 (F_IO_2 = 0.16 or 0.12).

Pulmonary $\dot{V}O_2$ was measured breath-by-breath using a flow meter and gas analyser system (Aeromonitor AE-300S; Minato Medical Science, Osaka, Japan). Lactate threshold (LT) was estimated non-invasively from combined ventilatory and gas exchange criteria (e.g. [2]). Deoxygenation of the right quadriceps was quantified by multi-channel TRS-NIRS at three muscle sites: the distal (VL_d) and proximal (VL_p) regions of the vastus lateralis, and the mid region of the rectus femoris (RF) (TRS-20, Hamamatsu Photonics KK, Hamamatsu, Japan). Each TRS-20 probe (consisting of a detector fixed at 3 cm from the emitter) provided pico-second light pulses at three different wavelengths (760, 795, and 830 nm) to measure (in micromoles) absolute muscle deoxygenation ([deoxy(Hb+Mb)]; here termed [HHb]), oxygenation ([oxy(Hb+Mb)]; here termed [HbO_2]), and total haemoglobin concentration ([Hb+Mb]; [Hb_{tot}]) at an output frequency of 0.5 Hz. Tissue oxygen saturation ([HbO_2]/[Hb_{tot}]; S_tO_2 %) was also calculated. To account for the influence of adipose tissue thickness (ATT) on the absolute haem concentrations, a linear regression was applied to the relationship between resting [Hb_{tot}] and ATT ([Hb_{tot}] = -21.4 · (ATT) + 220; r^2=0.77; P<0.001), and data were normalized to an ATT of zero as previously described [2].

Variables at baseline, LT, and peak exercise were determined from the average of 30 s. Data are presented as mean±SD. Differences in NIRS variables were assessed by two-way repeated measures ANOVA (F_IO_2 x muscle region). Other data were assessed by one-way repeated measures ANOVA among F_IO_2 conditions. Post hoc Bonferroni corrected t-tests determined the location of the differences where appropriate. The effect of F_IO_2 on spatial heterogeneity of [HHb] was determined by the coefficient of variation (CV (%): 100*SD/mean of 3 muscle regions). Significance was accepted at p<0.05. Analyses were completed using SPSS v.16.0 (SPSS Inc., Chicago, IL, USA).

3 Results

Lactate threshold (26±6, 22±5, 20±3 ml/kg/min, at F_IO_2 of 0.21, 0.16, and 0.12, respectively) and pulmonary $\dot{V}O_{2peak}$ (50±8, 45±5, 37±4 ml/kg/min) were progressively reduced with hypoxia (each p<0.05). As expected, the work rate at LT and peak exercise was also reduced (111±29, 101±34, 91±17 W and 282±54, 262±45, 221±36 W, respectively; p<0.05). The change in absolute quadriceps deoxygenation in each F_IO_2 condition is presented in Fig. 1. There was a significant effect (p<0.05) of F_IO_2 on [HHb] at baseline (57±5, 61±5, 69±6 uM), at LT (67±4, 70±3, 80±5 uM), and at peak (92±8, 97±7, 101±11 uM). Interestingly, peak [Hb_{tot}] was also lower in hypoxia: 237±24, 230±23, 227±26 uM (p<0.001; Fig. 1), as was S_tO_2: 62±2, 59±2, and 57±3 % (p<0.001; Fig. 1). The spatial

heterogeneity in [HHb] for a representative subject within and between muscle regions across F_IO_2 conditions is presented in Fig. 2. The spatial variance of [HHb] was not different between F_IO_2 conditions: baseline (8±4, 7±5, 9±5 %), LT (9±4, 7±4, 9±4 %), and peak (13±6, 11±4, 13±8 %; Fig. 3). Thus, there was no association between [HHb] heterogeneity and indices of aerobic energy transfer in the different F_IO_2 conditions at baseline, LT, or peak power output.

4 Discussion

We previously demonstrated that a hypoxia-induced slowing of $\dot{V}O_2$ kinetics during moderate intensity exercise was associated with reduced [HHb] heterogeneity [2]. As such, we hypothesized here that [HHb] spatial distribution would also become more homogenous during RI exercise in hypoxia. This was based on the notion that hypoxia may modulate microvascular blood flow and limit the capacity to maintain heterogeneity in muscle $\dot{Q}O_2/\dot{V}O_2$ during exercise – coincident with reduced metabolic flexibility in hypoxic conditions. To our surprise, however, we found the spatial distribution of [HHb] was unaffected by hypoxia during RI exercise.

Absolute skeletal muscle deoxygenation was progressively increased with the severity of hypoxia during RI exercise, despite progressively reduced peak power output. This may be due to a lower absolute baseline $\dot{Q}O_2$, which requires an increased O_2 extraction during exercise, even if the exercise $\dot{Q}O_2$ increment ($\Delta\dot{Q}O_2/\Delta\dot{V}O_2$) is unaffected [4]; and/or due to a reduced exercise $\Delta\dot{Q}O_2/\Delta\dot{V}O_2$ response in hypoxia. The overall exercise-induced [HHb] increase was not different between conditions (~35 uM), suggesting that a baseline reduction in $\dot{Q}O_2$ in hypoxia may have predominated. Nevertheless, whichever the mechanism, the observed hypoxic modulation in microvascular blood volume (reduced peak [Hb_{tot}]) is consistent with an attenuated vasodilation in hypoxia, suggesting that a vasodilatory reserve exists in hypoxia that may limit aerobic energy production.

Hypoxia has been suggested to reduce NO bioavailabilty, which serves as an important metabolite for vasodilation [5]. It therefore remains possible that hypoxia may have limited NO function and thus vasodilation, which could have disrupted the balance between $\dot{Q}O_2$ and $\dot{V}O_2$. Evidence in humans showing NOS inhibition impairs forearm vasodilation during exercise to a greater degree in hypoxia compared to normoxia supports this notion [5]. However, whether hypoxia decreases or increases NO remains controversial [3,5]. Exercise intensity is also known to alter blood flow spatial distribution in quadriceps muscles [6], with higher compared to lower power output associated with a more heterogeneous response. This may be related to an altered contribution of various vasoregulatory metabolites at different exercise intensities or in different fibre types, and/or an increased distribution of blood flow towards newly recruited muscle fibres [6]. This may also help explain why we found no reduction in the spatial heterogeneity of [HHb] during RI exercise: The expected reduction in [HHb] heterogeneity (based on findings from moderate intensity cycling [2]) were

offset by an increased heterogeneity during high intensity exercise recruiting poorly perfused muscle regions [6]. Although the CV for [HHb] was not different between F_IO_2 conditions, it is estimated that, with 7 subjects, we had power to detect an ~3% difference in regional deoxygenation CV.

In conclusion, absolute regional skeletal muscle deoxygenation measured by multi-channel TRS-NIRS was greater at rest and at the limit of tolerance in hypoxia compared to normoxia. However, contrary to our hypothesis this was not associated with a reduction in the spatial heterogeneity of [HHb] within and between quadriceps muscles. Of interest, the peak [Hb_{tot}] was reduced at the limit of tolerance in hypoxia compared to normoxia, implicating a vasodilatory reserve exists in hypoxia. This is consistent with the notion that hypoxia reduces exercise vasodilation and modulates the mechanisms linking $\dot{Q}O_2$ and $\dot{V}O_2$ that contribute to reduced metabolic flexibility.

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References

1. Koga S, Rossiter HB, Heinonen I et al (2014) Dynamic heterogeneity of exercising blood flow and O2 utilization. Med Sci Sports Exerc 46: 860-76.
2. Bowen TS, Rossiter HB, Benson AP et al (2013) Slowed oxygen uptake kinetics in hypoxia correlate with the transient peak and reduced spatial distribution of absolute skeletal muscle deoxygenation. Exp Physiol 98: 1585-96.
3. Ferreira LF, Hageman KS, Hahn SA et al (2006) Muscle microvascular oxygenation in chronic heart failure: role of nitric oxide availability. Acta Physiol (Oxf) 188: 3-13.
4. Benson AP, Grassi B, Rossiter HB (2013) A validated model of oxygen uptake and circulatory dynamic interactions at exercise onset in humans. J Appl Physiol 115: 743-55.
5. Casey DP, Walker BG, Curry TB et al (2011) Ageing reduces the compensatory vasodilation during hypoxic exercise: the role of nitric oxide. J Physiol 589: 1477-88.
6. Heinonen I, Sergey VN, Kemppainen J et al (2007) Role of adenosine in regulating the heterogeneity of skeletal muscle blood flow during exercise in humans. J Appl Physiol 103: 2042-2048.

Figure Legends

Fig 1. Deoxyhaemoglobin ([HHb]), total haemoglobin ([Hb_{tot}], and tissue saturation (S_tO_2) during ramp-incremental exercise in different F_IO_2 conditions (0.21, 0.16, 0.12). **A**) NIRS variables in the *vastus lateralis* (proximal region) from a representative subject; data are a 30 s average. **B**) Group NIRS variables (mean±SD) averaged across three muscle regions (*rectus femoris* and proximal and distal regions of the *vastus lateralis*). *$p<0.05$, significant effect of F_IO_2. Dashed vertical line represents start of ramp-incremental exercise.

Fig 2. Quadriceps deoxygenation ([HHb]) during ramp-incremental exercise in different F_IO_2 conditions (0.21, 0.16, 0.12) in a representative subject. Deoxygenation was measured in the

rectus femoris (RF) and proximal and distal regions of the *vastus lateralis* (VL_p and VL_d). Dashed vertical lines are the start and end of ramp-incremental exercise.

Fig 3. Mean coefficient of variation (CV) in quadriceps deoxygenation ([HHb]) during ramp-incremental exercise in different F_IO_2 conditions (0.21, 0.16, 0.12). CV values were determined from the spatial heterogeneity among the *rectus femoris* and proximal and distal regions of the *vastus lateralis*.