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Research article

The contribution of "resting" body muscles to the slow component of pulmonary oxygen uptake during high-intensity cycling

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Abstract
Oxygen uptake (VO_2) kinetics during moderate constant-workrate (WR) exercise (>lactate-threshold (θ_L)) are well described as exponential. Above θ_L, these kinetics are more complex, consequent to the development of a delayed slow component (VO_2sc), whose aetiology remains controversial. To assess the extent of the contribution to the VO_2sc from arm muscles involved in postural stability during cycling, six healthy subjects completed an incremental cycle-ergometer test to the tolerable limit for estimation of θ_L and determination of peak VO_2. They then completed two constant-WR tests at 90% of θ_L and two at 80% of Δ (difference between θ_L and VO_{2peak}). Gas exchange variables were derived breath-by-breath. Local oxygenation profiles of the vastus lateralis and biceps brachii muscles were assessed by near-infrared spectroscopy, with maximal voluntary contractions (MVC) of the relevant muscles being performed post-exercise to provide a frame of reference for normalising the exercise-related oxygenation responses across subjects. Above supra-θ_L, VO_2 rose in an exponential-like fashion ("phase 2), with a delayed VO_2sc subsequently developing. This was accompanied by an increase in [reduced haemoglobin] relative to baseline (Δ[Hb]), which attained 79 ± 13 % (mean, SD) of MVC maximum in vastus lateralis at end-exercise and 52 ± 27 % in biceps brachii. Biceps brachii Δ[Hb] was significantly correlated with VO_2 throughout the slow phase. In contrast, for sub-θ_L exercise, VO_2 rose exponentially to reach a steady state with a more modest increase in vastus lateralis Δ[Hb] (30 ± 11 %); biceps brachii Δ[Hb] was minimally affected (8 ± 2 %). That the intramuscular O_2 desaturation profile in biceps brachii was proportional to that for VO_2sc during supra-θ_L cycle ergometry is consistent with additional stabilizing arm work contributing to the VO_2sc.

Key words: Muscle oxygenation, near infrared spectroscopy, oxygen uptake kinetics, arm exercise.

Introduction

Following an initial "cardiodynamic" phase of response, the "phase 2" or "fundamental" pulmonary oxygen uptake (VO_2) kinetics for moderate-intensity constant-workrate (WR) cycle-ergometer exercise (i.e. below the lactate threshold (θ_L)) are well described as mono-exponential (e.g. Cerretelli and di Prampero, 1987; Henry and DeMoor, 1956; Grassi et al., 2003; Özyener et al., 2001; Whipp, 1972; Whipp et al., 1982):

$$\Delta VO_{2\,(t)} = \Delta VO_{2\,(ss)} \cdot (1 - e^{-(t-\delta)/\tau}) \quad (1)$$

where $\Delta VO_{2\,(ss)}$ is the steady-state increment or "gain", $\Delta VO_{2\,(t)}$ is the VO_2 increment at time t, and δ is a delay term that is a consequence of (but not necessarily equal to) the limb-to-lung vascular transit time (Whipp et al., 1982; 2002).

Above θ_L, however, the VO_2 kinetics become more complex (e.g. Barstow and Molé, 1991; Grassi et al., 2003; Linnarsson, 1974; Özyener et al., 2001; Paterson and Whipp, 1991; Poole et al., 1991; Whipp et al., 2005; Whipp and Wasserman, 1972). This reflects the superimposition on the fundamental component [with a τ and projected asymptotic gain similar to that of moderate exercise (Barstow and Molé, 1991; Özyener et al., 2001)] of an apparently delayed and slowly-developing "slow component" of response (VO_2 sc) which delays, or even prevents, the acquisition of an eventual steady state. As a result, the actual VO_2 achieved at end-exercise exceeds that predicted from the projected sub-θ_L VO_2-WR relationship, such that the overall gain ($\Delta VO_2/\Delta WR$) for cycle ergometry can be markedly increased from the normal 9-11 ml·min^{-1}·watt^{-1} to values of 14 ml·min^{-1}·watt^{-1} or more.

The source(s) of this "excess" VO_2 remains controversial (reviewed in Jones and Poole, 2005; Whipp et al., 2005). It has been argued that the exercising muscles themselves provide the major contribution (Poole et al., 1991; Rossiter et al., 2002), through mechanisms variously involving: the serial recruitment of energetically-inefficient type IIb fibres having a high VO_2 gain; acidosis, possibly via enhanced O_2-unloading from haemoglobin (the Bohr shift); the effects of increased levels of circulating catecholamines; and increased muscle temperature (Q_{10} effect). However, extra-muscular mechanisms have also been proposed, such as the O_2 cost of respiratory and cardiac work when ventilation and cardiac output are high, and the O_2 cost of extra "unmeasured" work. Of these, it is the latter that we chose to investigate.

During cycle ergometry, it is not uncommon for subjects to pull forcefully on the handle-bars at high work rates in an attempt to stabilise the body so that the required force output can be generated with the legs (e.g. Baker et al., 2001; 2002; Whitt and Wilson, 1989). It has been speculated that this has the potential to make a significant contribution to the VO_2 sc (reviewed in Jones and Poole, 2005; Whipp et al., 2005), although this has not been studied to date. To directly measure the O_2 consumption of the involved arm musculature is fraught with technical difficulties, however, because of the invasive procedures required. A useful non-invasive expedient is provided by the technique of transcutaneous near-infrared spectroscopy (NIRS) (Chance et al., 1992; Elwell, 1995;

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Mancini et al., 1994; Özyener, 2002), which has been widely used in constant-WR and incremental cycle ergometry (e.g. Belardinelli et al 1995; Bhambhani et al., 1998; Costes et al., 1996; DeLorey et al., 2007; Ferreira et al., 2005; Grassi et al., 2003; Wilson et al 1989) and arm exercise (e.g. Bhambhani et al., 1998; Mancini et al., 1994). Muscle oxygenation status in the region undergoing NIR interrogation (i.e. muscle and blood) is reflective of the corresponding muscle mean-capillary and muscle tissue O_2 contents, and thus the balance between local vascular O_2 delivery (i.e. the product of muscle blood flow and arterial O_2 content) and local O_2 consumption. Therefore, changes in the combined concentrations of oxyhaemoglobin and oxymyoglobin in the region of interest (termed, for convenience, $\Delta[HbO_2]$, as [myoglobin] is small compared to [haemoglobin]) are indicative of changes in tissue oxygenation status; similarly, changes in the combined concentrations of reduced haemoglobin and myoglobin (termed, for convenience, Δ[Hb]) are indicative of changes in tissue deoxygenation status.

We therefore hypothesised that constant-WR cycle ergometry at work rates sufficiently high to elicit a VO_2 sc would be associated with a contribution from the arms which would be manifest as a progressive deoxygenation (expressed as an increasing Δ[Hb]) in the interrogated arm musculature as the exercise proceeded. This would be expected to be absent for moderate work rates, where no VO_2 sc should be demonstrable.

Methods

Subjects and procedures

Six healthy subjects (age 33.7 ± 12.3 yr, height 1.79 ± 0.04 m, weight 79.8 ± 5.6 kg, peak VO_2 (VO_{2peak}) 45.83 ± 10.52 ml.kg^{-1}.min^{-1}) provided written informed consent to participate in the study. The study was approved by the St. George's Hospital Research Ethics Committee; procedures were conducted in accordance with the Declaration of Helsinki. The subjects were first familiarised with the equipment, procedures and laboratory personnel; and were requested to refrain from participating in strenuous exercise for the 24 hr prior to each testing session.

Exercise protocols

All tests were performed on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, NL), each on a different day for a given subject. An incremental ramp test (15 Watts·min^{-1}) to the limit of the tolerance was first completed in order to establish (1) VO_{2peak}, as the mean for an integral number of breaths over the final 20 s of the incremental phase; and (2) the estimated lactate threshold (θ_L), using standard ventilatory and gas exchange criteria (Beaver et al., 1973; Whipp et al., 1986). Each subject then completed four constant-WR tests: two sub-θ_L tests (90% of θ_L) of 6 min duration (i.e. sufficient to attain a steady state); and two supra-θ_L tests (80% of Δ), where Δ is the difference between θ_L and VO_{2peak}, for 15 min or to the limit of tolerance, whichever was the sooner (i.e. normalising work rates to the very-heavy intensity domain above critical power (e.g. Özyener et al., 2001; Whipp et al., 2005)). All tests were preceded by at least 4 min at 20 W, and followed by a 20 W recovery phase of at least 6 min.

Equipment

Pulmonary gas exchange and heart rate

The equipment and calibration procedures have been described in detail previously (Ozyener et al., 2011). The subjects breathed through a mouthpiece connected to a low-dead space, low resistance turbine volume transducer (Interface Associates, Irvine, CA, USA) for measurement of inspiratory and expiratory airflow and volume. Respired gas was continuously sampled from the mouthpiece and analysed by a quadrupole mass spectrometer (QP9000, Morgan Medical, Gillingham, UK) for [O_2], [CO_2] and [N_2]. Following analogue-to-digital conversion, the electrical signals from these devices were sampled and digitised for breath-by-breath determination of ventilatory and gas exchange variables (Beaver et al., 1973; Jenkins et al., 1989). Arterial O_2 saturation (Biox 3745, Ohmeda, Louisville, USA) and heart rate (Quest, Burdick, Washington, USA) were measured throughout.

Near-infrared spectroscopy

The equipment and calibration procedures have been described in detail previously. A NIR spectrometer (Hamamatsu NIRO 500, Hamamatsu Photonics KK, Japan) was used to monitor continuously the local intramuscular oxygenation profile in arm (biceps brachii) and leg (vastus lateralis) muscles as $\Delta[HbO_2]$, Δ[Hb] and their algebraic sum, the change in total [haemoglobin] (Δ[HbT]).

The transmitting and receiving optodes were positioned 4 cm apart in a rigid, optically-dense plastic holder placed on the surface of the right (i.e. dominant) leg along the long axis of the vastus lateralis muscle, at mid-thigh level, or on the surface of the right (i.e. dominant) arm along the long axis of the biceps brachii muscle at its midpoint. The light source was provided by four laser diodes (wavelengths: 776, 826, 845 and 905 nm), and was pulsed at 53 min^{-1}, i.e. lower than the rotation of the pedals (>60 rpm) to avoid interference. Changes from the resting baseline of [HbO2] (Δ[HbO2]), [Hb] (Δ[Hb]) and total [Hb] (Δ[HbT], = Δ[HbO2] + Δ[Hb]) were derived every 500 ms from the incident and transmitted light intensities and the relevant specific extinction coefficients and optical path length [see Elwell (1995) for details].

At the end of each test, the subject moved to a chair to perform a maximal voluntary isometric contraction (MVC) of the vastus lateralis or biceps brachii, as appropriate. This was obtained by the subject either pushing his right foot maximally against the floor (knee angle maintained close to 90°) or maximally pulling up against a low-compliance rubber band with his flexed right arm (elbow angle maintained close to 90°) to the limit of tolerance. The resulting maximal $\Delta[HbO_2]$, Δ[Hb] and Δ[HbT] excursions provided a frame of reference for normalising the $\Delta[HbO_2]$, Δ[Hb] and Δ[HbT] responses occurring during the constant-WR tests, in order to allow for inter-experiment variability in factors such optode placement and NIR signal intensity (Belardinelli et al., 1995; Chance

et al., 1992).

Data analysis and statistics

The breath-by-breath VO_2 data were edited to eliminate occasional breaths triggered by, for example, swallows, coughs or sighs which were considered to be uncharacteristic of the underlying physiological response; only breaths > 4 SD from the local mean were excluded (Lamarra et al., 1987). The baseline VO_2 was taken as the mean VO_2 for an integral number of breaths over the last 60 s of the 20 W baseline. The raw NIRS data were averaged over 5 s intervals (i.e. quasi breath-by-breath).

We did not attempt to formally partition the VO_2 or NIRS responses into their kinetic components (e.g. Barstow and Molé, 1991; Ferreira et al., 2005; Grassi et al., 2003; Özyener et al., 2001; Paterson and Whipp, 1991) as the low signal-to-noise ratio for paired transitions (owing to breath-to-breath variability) precluded an acceptable level of statistical confidence (Lamarra et al., 1987) and because of concerns about the most appropriate model fit to employ for the supra-θ_L exercise responses (see Whipp and Rossiter, 2005 for discussion). A simpler index of the magnitude of the "slow component" responses was therefore taken: the response increment from minute 3 of the exercise (del_2) and end-exercise (t_{end}); based on the widespread demonstration that the onset of the VO_2sc, while delayed, is typically discernible within 3 min of exercise onset (e.g. Barstow et al., 1996; Paterson and Whipp, 1991).

Relationships between intra-muscular oxygenation variables and the VO_2sc were studied using linear regression analysis and correlation coefficient techniques. These relations were considered significant if $p < 0.05$. Dispersion about mean values is expressed as ± standard deviation (SD), unless otherwise specified.

Results

We chose to use the change in the reduced [haemoglobin], Δ[Hb], as being best reflective of the changing intramuscular oxygenation status in the region of interest, as it is independent of local blood volume (and flow) changes in conditions where the arterial O_2 content is normal and levels of [Hb] are therefore trivial (De Blasi et al. 1994; Ferrari et al. 1997). In contrast, the oxygenated [haemoglobin], Δ[HbO_2], is highly volume-dependent, being influenced by changes in blood volume resulting from haemoconcentration and/or altered perfusion, and is therefore not an accurate reflection of O_2 extraction per se. In the absence of significant haemoconcentration, changes in blood volume can thus be visualised in the Δ[HbT] signal: when there are no such changes, the Δ[Hb] and Δ[HbO_2] responses will mirror each other (i.e. Δ[HbT] = 0). However, when blood volume increases, O_2 is brought into the field in combination with Hb. Consequently, Δ[HbO_2] is subject to two competing effects: a primary muscle-metabolic extraction (serving to decrease Δ[HbO_2]) and a secondary hydraulic-related delivery (serving to increase Δ[HbO_2]). Whether Δ[HbT] increases or decreases will depend on the relative magnitudes of these opposing effects.

Sub-θ_L exercise

Vastus lateralis oxygenation: Representative VO_2 and leg muscle (vastus lateralis) oxygenation profiles during a single bout of sub-θ_L constant-WR cycle ergometry are presented in Figure 1 (subject 1). VO_2 increased at exercise onset without discernible delay, rising in an exponential-like fashion to attain a steady state within ~ 2 min (e.g. Cerretelli and di Prampero, 1987; Grassi et al., 2003; Henry and DeMoor, 1956; Özyener et al., 2001; Whipp, 1972; Whipp et al., 1982). Δ[Hb] rose even more abruptly, stabilising within ~ 30 s (e.g. Ferreira et al., 2005; DeLorey et al., 2007; Grassi et al., 2003).

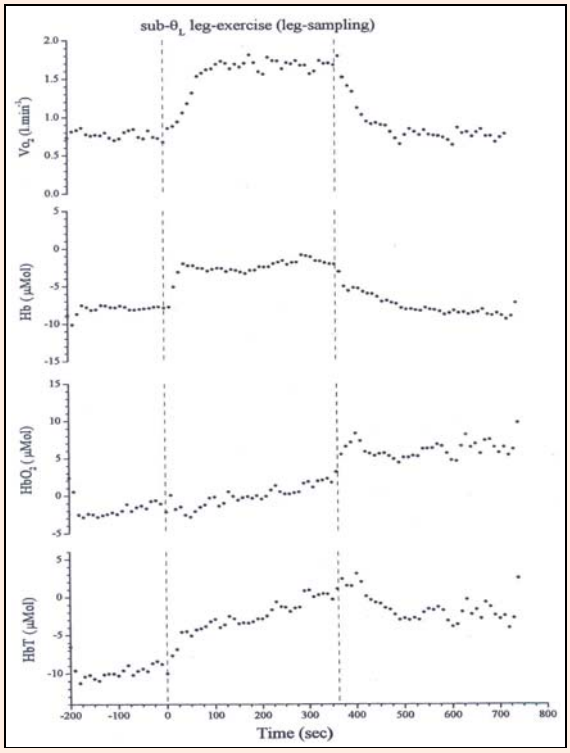

Figure 1. An example (subject 1) of the oxygen uptake (VO_2) and vastus lateralis NIRS responses to constant-WR sub-θ_L cycle ergometry. Δ[Hb], Δ[HbO_2] and Δ[HbT] represent the changes in [reduced haemoglobin], [oxyhaemoglobin] and [total haemoglobin], respectively (see text for details). The dashed vertical lines indicate the start and end of the exercise bout.

In contrast, while Δ[HbO_2] evidenced a rapid decrease at exercise onset, this was transient being followed by an increase which, in this subject, tended to continue throughout the exercise while, in others, it stabilised (e.g. DeLorey et al., 2007; Grassi et al., 2003). Consequently, Δ[HbT] increased abruptly and then more slowly as the exercise progressed. These data clearly illustrate the dependency of Δ[HbO_2] in the region of interest on Δ[HbT], in contrast to Δ[Hb].

Biceps brachii oxygenation: The corresponding arm muscle (biceps brachii) oxygenation profiles during the same bout of sub-θ_L constant-WR cycle ergometry are presented in Figure 2 (the VO_2 response from Figure 1 is reproduced to provide a frame of reference). It is evident

that Δ[Hb], Δ[HbO_2] and Δ[HbT] each demonstrated minimal change from their 20W baseline levels over the duration of the leg exercise bout. This was a consistent finding among all subjects.

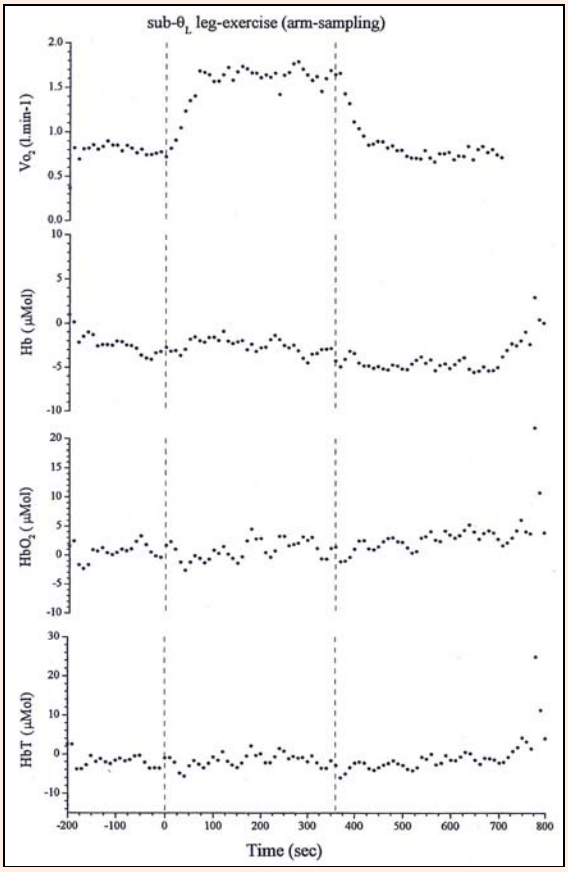

Figure 2. For the same subject presented in Figure 1, the biceps brachii NIRS responses to constant-WR sub-θ_L cycle ergometry (the VO_2 response is displayed again for reference).

Supra-θ_L exercise

Vastus lateralis oxygenation: An example of the VO_2 and leg muscle (vastus lateralis) oxygenation profiles during a single bout of supra-θ_L constant-WR cycle ergometry is presented in Figure 3 (subject 1). VO_2 increased at exercise onset without discernible delay and to a more marked degree than for the sub-θ_L exercise (Figure 1), again initially rising in an exponential-like fashion but then demonstrating a slow progressive increase (slow component) which was sustained for the remaining duration of the exercise (e.g. Grassi et al., 2003; Barstow & Molé, 1991; Linnarsson, 1974; Özyener et al., 2001; Paterson & Whipp, 1991; Poole et al., 1991; Whipp & Wasserman, 1972; Whipp et al., 2005). Δ[Hb] rose more abruptly, and to a greater extent than for sub-θ_L exercise (Figure 1), then also continuing to rise more slowly throughout the exercise (e.g. Ferreira et al., 2005; Grassi et al., 2003). The Δ[Hb] increment between baseline and end-exercise was 22.4 µM or 59 % of the MVC-induced response (Figure 3; see Methods). For the group as a whole, this MVC-normalised Δ[Hb] increment averaged 79 ± 13 % (range: 62-93 %), and was significantly greater than the 30 ± 11 % (range: 14-46 %) seen for the sub-θ_L exercise (Figure 4).

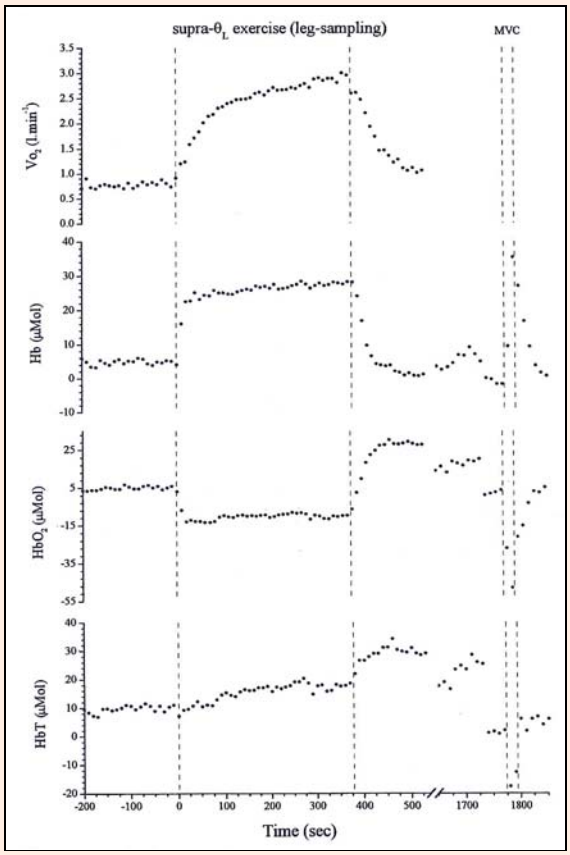

Figure 3. For the same subject presented in Figure 1, the VO_2 and vastus lateralis NIRS responses to constant-WR supra-θ_L cycle ergometry. The NIRS responses to a subsequently-performed maximal voluntary contraction (MVC) of the vastus lateralis are shown at the right (see text for further details).

In contrast to the sub-θ_L exercise (Figure 1), Δ[HbO_2] decreased rapidly at exercise onset with no clear tendency to increase substantially later in the bout (Figure 3). As the Δ[Hb] increase was slightly greater than the Δ[HbO_2] decrease, Δ[HbT] increased slowly and progressively throughout the exercise.

Biceps brachii oxygenation: The corresponding arm muscle (biceps brachii) oxygenation profiles during the same bout of supra-θ_L constant-WR cycle ergometry are presented in Figure 5 (the VO_2 response from Figure 3 is reproduced to provide a frame of reference). In contrast to the sub-θ_L responses (Figure 2), Δ[Hb] increased throughout the exercise and Δ[HbO_2] decreased. The resulting Δ[HbT] response was therefore an initial modest decline, followed by a slow and also modest increase for the remainder of the exercise.

The Δ[Hb] increment between baseline and end-exercise was 17.6 µM or 42 % of the MVC-induced response (Figure 5; see Methods). For the group as a whole, this MVC-normalised Δ[Hb] increment averaged 32 ± 27 % (range: 17-85 %), significantly greater than the 8 ± 2 %

(range: 5-12 %) observed for the sub-θ_L exercise (Figure 4).

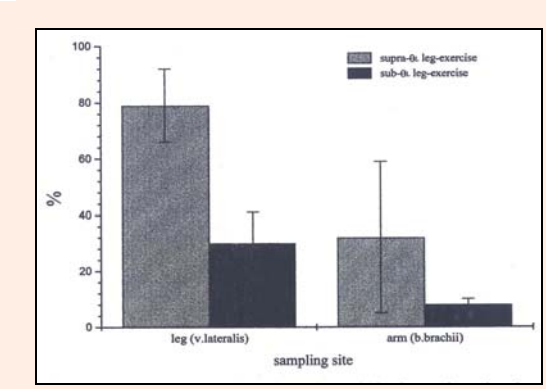

Figure 4. Normalised vastus lateralis (left) and biceps brachii (right) Δ[Hb] responses (expressed as % of MVC values) for six subjects to constant-WR supra-θ_L (light shading) and supra-θ_L (dark shading) cycle ergometry. See text for details.

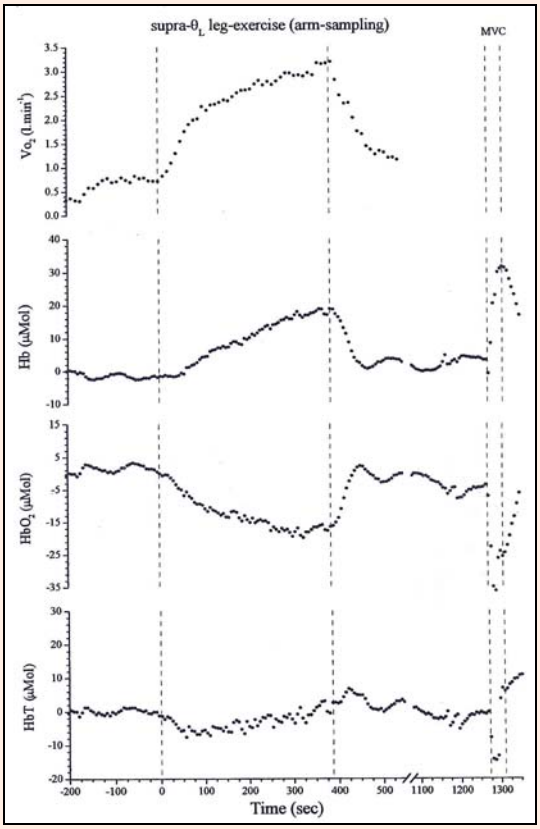

Figure 5. For the same subject presented in Figure 1, the biceps brachii NIRS responses to constant-WR supra-θ_L cycle ergometry (the VO_2 response is displayed again for reference). The NIRS responses to a subsequently-performed maximal voluntary contraction of the biceps brachii are shown at the right.

Correlation between VO_2sc and Δ[Hb]: It should be noted that the biceps brachii NIRS response profile presented in Figure 5 was not seen in all of our subjects. That is, in some instances, there was little obvious increase in Δ[Hb] during the supra-θ_L cycling, despite a VO_2sc being evident, which might suggest that these subjects relied far less on their upper-body musculature during the supra-θ_L exercise. An example of this response is shown in Figure 6.

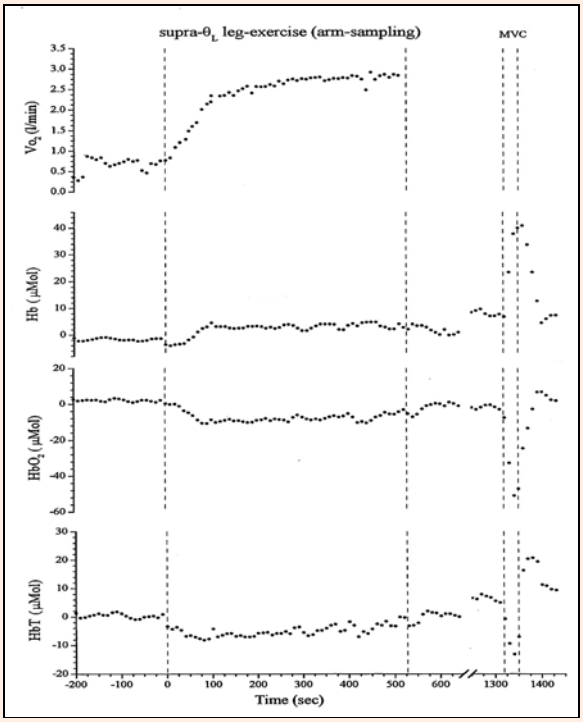

Figure 6. For subject 6, the biceps brachii NIRS responses to constant-WR supra-θ_L cycle ergometry (the VO_2 response is displayed again for reference). The NIRS responses to a subsequently-performed maximal voluntary contraction of the biceps brachii are shown at the right.

It was of interest, therefore, to explore the correlation between the magnitudes of the VO_2sc and Δ[Hb] responses across our subjects. The estimated VO_2sc (i.e. the VO_2 increment between the onset of the slow component (t = del_2) and end-exercise (t_{end})) was found to be significantly proportional to the magnitude of the Δ[Hb] response, whether this was expressed as the response over the entire supra-θ_L exercise bout (Δ[Hb] (t_{end}-t_0)) (Figure 7, upper panel: slope (standard error) = 0.004 (0.001), r = 0.83) or that confined to the slow-component region (Δ[Hb] (t_{end}- del_2)) (Figure 7, lower panel: slope (standard error) = 0.003 (0.001), r = 0.68). Reinforcing this finding was the demonstration of a significant correlation between VO_2 and Δ[Hb] for four out of six subjects when the entire data set within the slow-component region was analysed (Table 1); for the vastus lateralis interrogation, this occurred in five out of six subjects.

Discussion

This study has demonstrated that progressive deoxygenation (i.e. increasing Δ[Hb]) occurs in a NIRS-interrogated arm muscle (biceps brachii) during supra-θ_L constant-WR

cycle ergometry (Figure 5) for which a VO_2sc was consistently demonstrated, but not during sub-θ_L constant-WR cycle ergometry (Figure 3). Furthermore, the magnitude of the VO_2sc between subjects was found to be proportional to that of the biceps brachii Δ[Hb], both in terms of the values achieved at end-exercise (Figure 7) and their correlation throughout the slow-component phase (Table 1). These findings cohere with the forearm ergometric and electromyographic observations of Baker et al. (2001; 2002) who have suggested that pulling on the ergometer handlebars maintains the body's centre of mass at a constant height relative to the ergometer seat, thus optimising force generation on the pedals during leg extension. This allows higher peak power outputs to be generated (Baker et al., 2001; 2002) and, presumably, given high-intensity work rates to be sustained for longer periods.

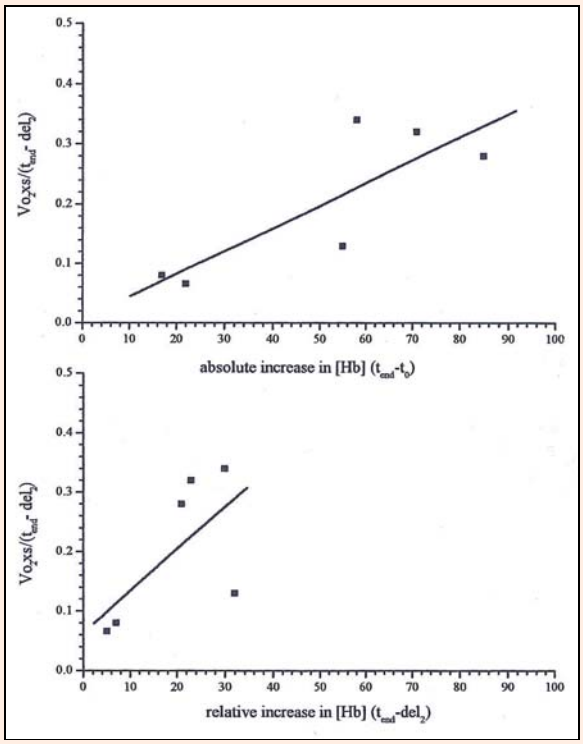

Figure 7. Upper panel – The VO_2sc, the VO_2 increment between the onset of the slow component (t = del_2) and end-exercise (t_{end}) (VO_2xs (t_{end} – del_2)), plotted against the biceps brachii Δ[Hb] response measured over the entire supra-θ_L work bout (Δ[Hb] (t_{end}-t_0)). Lower panel - VO_2xs (t_{end} – del_2) plotted against the biceps brachii Δ[Hb] response measured over the slow-component region (Δ[Hb] (t_{end}- del_2)) See text for details.

Our data suggest, therefore, that the metabolic cost of using the arms (and shoulder girdle) muscles to assist in stabilising the body for more effective force generation during high-intensity cycle-ergometer exercise has the potential to make an appreciable contribution to the time course and magnitude of the VO_2sc. This contention would seem to be at odds with earlier conclusions of Poole et al. (1991) that well over 80% of the VO_2sc during high-intensity cycle ergometry derives from oxygen consumption (QO_2) in the legs, precluding a major role for factors such as "auxiliary muscle work". However, there is the possibility that the contribution from the legs might have been overestimated: taking their Figure 1 as representative, a single final high QO_2value (possibly reflective of the final effort just before exhaustion) may have given undue weight to the fitting process.

Table 1. Individual correlation coefficients (r) between VO_2 and deoxygenated haemoglobin (Δ[Hb]) throughout the "slow-component" region for supra-θ_L constant-workrate cycle-ergometer exercise.

	Vastus lateralis		Biceps brachii	
Subject No.	r	*P*	r	*P*
1	.60	<.001	.89	<.001
2	.61	<.005	.88	<.001
3	.75	<.001	.36	<.05
4	.62	<.008	-.44	.80
5	-.35	.24	.86	<.001
6	.40	<.005	.03	.85

Even taking this possible "distorting" influence into account, there is little question that the exercising legs make a dominant contribution to the VO_2sc during supra-θ_L constant-WR cycle ergometry (reviewed in Jones and Poole, 2005; Whipp et al., 2005). Thus, in addition, during knee-extensor exercise, the VO_2sc has been shown to be accompanied by a similar slow component of intramuscular [phosphocreatine] decrease (Rossiter et al., 2002); a finding that argues strongly for the contribution arising from a high phosphate cost of force production rather than a high O_2 cost of phosphate production (see also Bangsbo et al., 2001) - possibly arising from the serial recruitment of energetically-inefficient type IIb fibres (reviewed in Jones et al., 2005). Additional intramuscular contributions could arise from intramuscular acidosis (Zoladz et al., 1998) possibly via enhanced unloading of O_2 unloading from haemoglobin (Stringer et al., 1994), increased levels of circulating catecholamines, and increased muscle temperature.

Interestingly, our data also showed that the magnitude of the biceps brachii Δ[Hb] response over the slow-component region varied widely between subjects – from trivially small values (Figure 6) to appreciably significant (Figure 7) (Table 1). This raises some important practical aspects that might be borne in mind when designing cycle-ergometer studies of VO_2 kinetics in high-intensity exercise. That is, employing pre-experimental familiarisation of the subjects specifically with respect to cycling technique in order to ensure a stable and relatively "fixed" upper-body posture might be expected to minimise variable extraneous contributions from the arms and shoulder girdle to the kinetics not only of the VO_2sc but of the entire VO_2 response.

It is important to recognise that the intramuscular Δ[Hb] response is not directly reflective of the underlying QO_2 response, as it captures only the "extraction" component. We were unable to monitor QO_2 in either leg or arm muscle, because of the technical complexities associated with such invasive procedures. Ferreira et al. (2005) have proposed a NIRS-based method for estimating muscle capillary blood flow (Q) kinetics during exercise, based on the recognition that the Δ[Hb] response reflects the

QO_2 to-Q ratio in the interrogated muscle capillaries (DeLorey et al., 2007; Grassi et al., 2003). While this approach appears to have promise for examining Q kinetics in moderate-intensity exercise, where assumptions regarding the exponentiality of the phase 2 VO_2 kinetics are reasonably robust, this is not so for an exponential characterisation of the VO_2sc (for discussion, see Whipp and Rossiter, 2005). An alternative approach, also highly assumption-laden, is to infer the associated behaviour of Q from the temporal profile of the Δ[HbT] response, which depends not only on the volume of blood within the region of interrogation but also on whether there are any concomitant exercise-related trans-capillary fluid fluxes. Because of this uncertainty, we chose not to pursue this.

A fundamental assumption of our study is that the vastus lateralis and the biceps brachii were sufficiently representative of the involved "leg" and "arm" musculature, respectively. The vastus lateralis is a major muscle contributing to force generation during cycle-ergometry and has been extensively studied with respect to its structure, biochemistry, metabolism, contraction, blood supply and exercise response (e.g. Saltin and Gollnick, 1983) and also with respect to NIRS function (e.g. Belardinelli et al 1995; Bhambhani et al., 1998; Costes et al., 1996; Grassi et al., 2003; DeLorey et al., 2007; Ferreira et al., 2005; Wilson et al 1989). Recent developments in NIRS technology allowing multi-site recordings have shown that desaturation profiles for vastus medialis are similar to those in vastus lateralis during incremental cycle ergometry, although the rectus femoris desaturation tended to be less marked; differences that were tentatively ascribed to differences in muscle fibre-type recruitment (Chin et al., 2011). The biceps brachii was chosen as it was likely to have a major involvement in any upper-body accessory activity during high-intensity cycle-ergometry. For each of these muscles, we were able to secure a physiological "calibration" in all our subjects, i.e. a reproducible MVC with a reproducible NIRS response profile (e.g. Figures 3 and 5).

A further assumption relates to the region of the muscle chosen for interrogation: were the regions of the vastus lateralis and biceps brachii chosen for NIRS interrogation representative of the muscles as a whole? With regard to vastus lateralis, the NIRS optodes were placed over the mid portion of the muscle, which Belardinelli et al. (1995) have argued is recruited at any time when the whole muscle is activated. Also, Boone et al. (2010) have demonstrated qualitatively and quantitatively similar profiles of muscle desaturation at proximal and distal sites of vastus lateralis.

With regard to the NIRS technique itself, several points require discussion. Firstly, in order to minimize undue variation between measurement periods, care was taken to ensure that the optode assembly was secured to the skin overlying the muscle surface, so as to minimise movement and therefore extraneous influences on NIR light path length. However, movement of the muscle tissue itself is harder to prevent, although these effects were minimised as far as possible by ensuring that optode placement and cycling cadence were similar between tests. Secondly, as NIR light is almost completely absorbed by large blood vessels, it is assumed that the NIRS signals are primarily related to changes in oxygenation occurring within interrogated small intramuscular vessels and capillaries (Chance et al., 1992). And it has been argued that the oxygenation status of overlying cutaneous blood vessels normally has little effect on NIR signal intensity (Mancini et al., 1994). Finally, there is the possibility that subcutaneous adiposity influenced the NIR signal characteristics, as Chin et al. (2011) have demonstrated using Doppler ultrasound. However, we did not have access to this equipment and therefore can only speculate on the magnitude of this effect: what we can say is that, as our subjects were all lean, we might not expect this to be a major limitation.

Finally, the translation of biceps brachii desaturation profiles during high-intensity cycle ergometry into energetic equivalents for force generation should be undertaken with caution: such inferences should not be drawn *a priori* from responses of muscles such as the vastus lateralis. That is, factors such as a more marked heterogeneity in blood flow distribution, a smaller diffusing area and greater diffusing distances in arm musculature (e.g. Calbet et al., 2005) could contribute to the efficiency (or otherwise) of the eventual "processing" of the extracted oxygen.

Conclusion

The intra-muscular oxygenation profile of the arm (biceps brachii muscle) during constant-WR high-intensity cycle ergometry exercise evidenced considerable desaturation, in concert with the presence of a VO_2 slow component. This suggests that additional work done by the arm muscles to stabilise the body at high work rates may contribute to the VO_2 slow component, although the question of "How much?" remains to be determined.

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Key points

- The source(s) of the "slow component" component of pulmonary oxygen uptake kinetics (VO_2sc) associated with high-intensity exercise remains the source of debate.
- Noninvasive interrogation techniques of the biceps brachii muscle during cycle ergometry suggest that "resting" muscles, such as those of the arms and shoulder girdle, may contribute to the VO_2sc, through stabilising actions (e.g. more forceful pulling on the ergometer handlebars).
- The quantitative contribution of such extra "resting"-muscle work awaits precise determination.

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