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ORIGINAL ARTICLE

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Muscle oxygenation kinetics at the onset of exercise do not depend on exercise intensity

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Abstract The purpose of this study was to determine whether the onset kinetics of muscle oxygenation in localized working muscle (mOxy) was affected by differences in exercise intensity. Five healthy male subjects exercised for 6 min at 125 W, 150 W, and 175 W, and 1 min at 300 W on a cycle ergometer. mOxy was estimated by near-infrared spectroscopy (NIRS) with a continuous wave photometer. The NIRS probe was positioned on the vastus lateralis muscle of the right leg. The relative change in mOxy was calculated from the relative change of the oxygenated hemoglobin (OxyHb) and deoxygenated hemoglobin (DeoxyHb) concentration from their resting values ([mOxy] = Δ[OxyHb]−Δ[DeoxyHb]). Assuming an exponential time course with time delay, the time constants of the mOxy were 5.7 (SD 2.2) s at 125 W, 5.6 (SD 1.9) s at 150 W, 6.0 (SD 2.2) s at 175 W, and 5.6 (SD 2.1) s at 300 W. The time delays of the mOxy were 6.7 (SD 4.2) s at 125 W, 8.6 (SD 1.6) s at 150 W, 6.4 (SD 3.0) s at 175 W, and 5.4 (SD 2.9) s at 300 W. The mean response times of the mOxy were 12.5 (SD 2.7) s at 125 W, 14.2 (SD 2.4) s at 150 W, 12.4 (SD 4.4) s at 175 W, and 11.0 (SD 3.1) s at 300 W. These results indicate that the kinetics of mOxy were not affected by differences in exercise intensity.

Keywords Near-infrared spectroscopy · Exercise intensity · Kinetics · Time constant

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Introduction

There exists substantial controversy as to whether muscle oxygen delivery ($\dot{Q}O_2$) or muscle mitochondrial oxygen demand determines the profile of pulmonary oxygen uptake ($\dot{V}O_2$) in the rest-to-exercise transition. Knowledge of what limits the speed of $\dot{V}O_2$ kinetics during the rest-to-exercise transition is important because awake humans rarely remain in a constant metabolic steady state for prolonged periods of time.

To address this issue, we adopted near-infrared spectroscopy (NIRS) for monitoring of the balance between muscle $\dot{V}O_2$ and muscle $\dot{Q}O_2$ in human the vastus lateralis muscle during cycling on-transition exercise. NIRS was first applied to the study of exercising skeletal muscle in humans by Chance et al. (1985). Over the past several years, NIRS has become widely used in research to non-invasively measure muscle tissue oxygen status. The commonly derived parameters from NIRS in humans are changes in oxyhemoglobin (OxyHb) and deoxyhemoglobin (DeoxyHb) in the tissue measured. It has been proposed that the muscle oxygenation (mOxy) calculated from these parameters indicates the balance between $\dot{Q}O_2$ and tissue $\dot{V}O_2$ (Chance et al. 1992).

It has been reported that the oxygen consumption of leg muscles increases in close relation to blood flow during steady-state levels of constant work-rate exercise (Vollestad et al. 1990), suggesting that the oxygen transport–consumption balance in the monitored muscle tissue is not only exercise intensity dependent but also exercise duration dependent. It is known that the level of mOxy decreases with exercise intensity (Bhambhani et al. 1997; Grassi et al. 1999). In addition, Hamaoka et al. (1998) showed that the kinetics of mOxy at the onset of exercise were associated with the relative occurrence of type I fibers in muscle tissue.

At the onset of constant-load exercise, ATP demand increases instantaneously. However, pulmonary $\dot{V}O_2$ follows a finite kinetic response. The kinetic feature of

this response and associated mechanistic interpretations remain controversial. To address this controversy, Behnke et al. (2001) adapted intravascular phosphorescence quenching techniques for measurement of spinotrapezius microvascular oxygen pressures. They found that the response profile of oxygen pressure of muscle was well fit by a time delay about 20 s followed by a monoexponential decline to its steady-state level. We hypothesized that similar kinetics can be observed in exercising muscle tissue in humans, and that these will provide evidence that muscle blood flow and therefore oxygen delivery do not limit $\dot{V}O_2$ kinetics at the onset of exercise under the conditions of this experiment. Previous studies have examined the kinetics of mOxy by NIRS during constant-load exercise (MacDonald et al. 1998; Burnley et al. 2002). However, there has been no study to determine whether the time constant of mOxy at the onset of exercise as measured by NIRS is affected by exercise intensity. Therefore, the purpose of our study was to examine the relationship between exercise intensity and the kinetics of mOxy in the femoral region (vastus lateralis muscle) during constant work-rate exercise on a cycle ergometer.

Methods

Subjects

This study included five healthy males ranging in age from 23 to 28 years [height 1.78 (SD 0.06) m, body mass 72.0 (11.6) kg]. Prior to the measurements, the purpose and methods of this study were explained and informed consent was obtained from all subjects. The mean value of maximal oxygen uptake per body mass ($\dot{V}O_{2max}$) was 51.9 (7.0) ml kg^{-1} min^{-1}.

Exercise protocol

All the subjects participated in four exercise trials on 4 separate days within 2 weeks: three 6-min trials at 125 W, 150 W, and 175 W, and one 1-min exercise trial at 300 W on a Monark cycle ergometer. The number of pedal rotations was 50 rpm. The flywheel was accelerated before each trial by an experimenter to avoid an extra power output at the onset of exercise and to overcome the moment of inertia of the ergometer's flywheel. The trials were randomly selected to avoid any ordering effect.

Pulmonary gas analysis

Breath-by-breath measurements of the $\dot{V}O_2$ and heart rate (HR) were obtained with a computerized system (K4b^2, Cosmed, Italy) at the three 6-min trials. The gas analysis system was checked immediately before each exercise trial with known reference gases and a syringe of the known volume. Care was taken to use flow rates similar to those of the subject's ventilation during the exercise trial. The breath-by-breath data were later reduced to 30-s stationary averages (Data Management Software, Cosmed) as described in Demarle et al. (2001). Fingertip capillary blood samples were collected before exercise and at the end of exercise to analyze lactate concentration.

Peripheral muscle oxygenation

During the exercise test, the kinetics of peripheral mOxy were measured in the vastus lateralis muscle using NIRS (BOM-L1TR, Omega Wave, Japan) (Kashima et al. 1992). This instrument uses three laser diodes (780, 810, and 830 nm), and calculates relative tissue levels of OxyHb, DeoxyHb, and total hemoglobin (TotalHb) according to the modified Beer–Lambert law. A probe, which consists of a light source and silicon, was positioned over the right vastus lateralis muscle midway between the trochanter major and condylus lateralis. The distance between the light source and the photodetector was 3 cm.

The method uses the known absorption properties of hemoglobin (Hb). In addition, the in vivo extinction coefficients of the two chromophores at each of the wavelengths must be known. A minimum of two wavelengths is necessary to solve an equation with two variables; we used the instruments using 780, 810, and 830 nm for the conversion to concentrations.

At each of the wavelengths, the absorbance is proportional to the product of the extinction coefficient of the chromophore (e), the concentration of the chromophore (c), and the path length of the light(l). The total absorbance (A) at one wavelength (n) is the sum of the absorbances of the different absorbing components:

$$\Delta A_n = (\Delta e_n(\text{OxyHb}) \times l \times c(\text{OxyHb})) + (\Delta e_n(\text{DeoxyHb}) \times l \times c(\text{DeoxyHb})) \quad (1)$$

From the extinction coefficients provided by Wray et al. (1988), the multiplication factors were obtained by means of linear interpolation and matrix inversion. Changes in concentrations of OxyHb and DeoxyHb are calculated by summation of the absorption changes from the start of monitoring (measured as optical density) multiplied by the above coefficients at each the wavelength. Changes in [TotalHb] are the sum of [OxyHb] and [DeoxyHb]. In as much as at present the l is unknown, the results are relative and expressed with the arbitrary units.

Changes in light absorption and Hb concentration were determined according to a modified version of the Beer–Lambert Law (Delpy et al. 1988), in which a differential path-length factor (DPF = 4.0) is incorporated to account for the light scattering in the muscle tissue. This instrument is a useful instrument for monitoring mOxy as reported in the previous study (Kawaguchi et al. 2001).

mOxy was calculated for the OxyHb and DeoxyHb values using the following formula based on the resting values:

$$\text{mOxy} = \Delta[\text{OxyHb}] - \Delta[\text{DeoxyHb}] \quad (2)$$

For the measurements, the distance between the incident point and the detector was 30 mm. The incident point and detector were fixed with elastic tape after shielding with a rubber sheet and vinyl. The data were inputed into a personal computer at a sampling frequency of 2 Hz via an A/D transducer (MacLab, AD Instruments).

Curve fitting for peripheral muscle deoxygenation

The mOxy values were then curve-fit to a mono-exponential plus delay using an iterative least-squares technique by means of a commercial graphing/analysis package (KaleidaGraph 3.1). For those instances where mOxy actually increased temporarily across the transition, the curve fit was performed on the data obtained as mOxy fell below the baseline values. The equations utilized were as follows:

$$\text{mOxy}(t) = \text{mOxy}(b) - \Delta\text{mOxy}(ss) * (1 - \exp(-(t - TD)/\tau)) \quad (3)$$

where mOxy(t), mOxy(b), and ΔmOxy(ss) designate mOxy at time t, baseline (i.e. pre-contraction) mOxy, and the decrease in mOxy from baseline to exercising steady state, respectively. TD is the time delay, and τ is the time constant of the response. The overall kinetics of the response were determined from mean response time; this was calculated by fitting the response data of mOxy to a

Table 1 The maximal oxygen uptake ($\dot{V}O_{2max}$), actual percentage of maximal oxygen uptake ($\%\dot{V}O_{2max}$), and calculated percentage of maximal oxygen uptake (*Calculated* $\dot{V}O_{2max}$) (300 W) for each subject

Subject no.	$\dot{V}O_{2max}$ (ml min^{-1})	$\%\dot{V}O_{2max}$ at 125 W	$\%\dot{V}O_{2max}$ at 150 W	$\%\dot{V}O_{2max}$ at 175 W	Calculated $\%\dot{V}O_{2max}$ at 300 W
1	3,477	58.5%	75.0%	87.5%	159.0%
2	3,809	58.6%	68.8%	79.3%	135.3%
3	3,661	60.5%	71.3%	83.1%	169.7%
4	3,393	59.2%	76.2%	83.3%	187.1%
5	3,300	61.7%	79.2%	90.2%	187.2%

monoexponential function that included single amplitude and τ, starting from the onset of the transition.

Statistical analysis

To examine statistically significant differences, the τ values of mOxy obtained during the exercise test between several exercise intensities were tested using a paired Student's *t*-test.

Results

The $\dot{V}O_2$ values at the last 1 min of the 6-min exercise were 2,105 (109) ml min^{-1} [59.7 (1.4)% $\dot{V}O_{2max}$] at 125 W, 2,654 (194) ml min^{-1} [75.3 (4.7)% $\dot{V}O_{2max}$] at 150 W, and 3,184 (134) ml min^{-1} [90.5 (7.0)% $\dot{V}O_{2max}$) at 175 W. The values for HR at the last 1 min of the 6-min exercise were 120 (17) beats min^{-1} at 125 W, 138 (17) beats min^{-1} at 150 W, and 164 (12) beats min^{-1} at 175 W. The blood lactate concentrations at the end of exercise were 2.9 (0.5) mmol l^{-1} at 125 W, 3.2 (1.1) mmol l^{-1} at 150 W, 5.2 (1.1) mmol l^{-1} at 175 W, and 9.0 (1.4) mmol l^{-1} at 300 W. At the end of the trial at 300 W, each subject was close to his volitional fatigue.

Table 1 shows the percentage of each subject's $\dot{V}O_{2max}$ at the chosen workloads.

The dynamics of mOxy at each exercise intensity are represented in Fig. 1. We found a slow component of mOxy at 175 W. There were no significant differences between the TD and τ among the four exercise intensities ($P > 0.05$) (Table 2). There were no significant differences in the mean response times for the mOxy kinetics among the four exercise intensities: 12.5 (2.7) s at 125 W, 14.2 (2.4) s at 150 W, 12.4 (4.4) s at 175 W, and 11.0 (3.1) s at 300 W, $P > 0.05$ (Table 2).

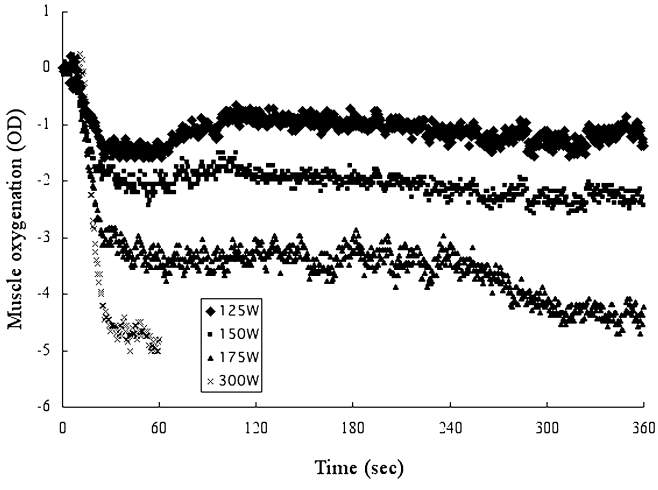

Fig. 1 Typical time course changes in muscle deoxygenation in one subject at four constant work-rate exercise sessions. *OD* Optical density

Discussion

The main finding of the present study was the absence of any significant differences in the kinetics for the mOxy at the onset of exercise among the broad range of exercise intensities.

The non-invasive method of NIRS is based on the principle that the light absorption characteristics of Hb and myoglobin in the near-infrared region changes depending on their oxygen saturation (Delpy and Cope 1997). The impossibility of obtaining quantitatively accurate values of tissue oxygenation represents an intrinsic limit of the optical methods that assume the homogeneity of examined tissue volumes, even by utilizing the most sophisticated quantitative algorithms of the intensity-modulated instruments. Thus it is impossible to compare the quantitative changes in mOxy with exercise intensity in separate exercise protocols without considering the blood volume and flow in the region of interest. However, it is possible to evaluate the relative changes in mOxy as used in the present study. In the present study, the relative changes of the mOxy kinetics parameters [τ, TD and mean response times (MRT) for mOxy] did not differ among the four exercise intensities in spite of the large differences in exercise intensities among the four cycling bouts. Moreover, we found that the mOxy kinetics were characterized by a definite TD. If $\dot{Q}O_2$ was the factor limiting muscle $\dot{V}O_2$ kinetics at exercise onset, one would expect an immediate and precipitous drop in mOxy. The lack of immediate changes of the balance between $\dot{V}O_2$ and $\dot{Q}O_2$ across the

Table 2 Time delay, time constant, and mean response time at several values of work-rate exercise for muscle deoxygenation. Values are means (SD)

	Work rate			
	125 W	150 W	175 W	300 W
Time delay (s)	6.7 (4.2)	8.6 (1.6)	6.4 (3.0)	5.4 (2.9)
Time constant (s)	5.7 (2.2)	5.6 (1.9)	6.0 (2.2)	5.6 (2.1)
Mean response time (s)	12.5 (2.7)	14.2 (2.4)	12.4 (4.4)	11.0 (3.1)

rest-to-exercise transition was observed in the rat skeletal muscle (Behnke et al. 2001).

mOxy could, in theory, be attributed to an accelerated fall of capillary–venular partial pressure of oxygen (Grassi et al. 1999). Near-infrared light-absorption changes in muscle reflect the changes in deoxygenation at the level of small blood vessels, capillaries, intracellular, and intercellular sites. It has been reported that when the oxygen supply is presumed not to be limiting, muscle oxygen uptake kinetics are constant across a wide range of power outputs in isolated muscles (Mahler 1985). In addition, Whipp and Mahler (1980) reported that under the condition of non-limiting oxygen delivery, the kinetics of muscle $\dot{V}O_2$ are determined by the mitochondrial content of contracting muscle. Therefore, there is a possibility that the constancy of the τ for mOxy kinetics might reflect the insufficient condition of oxygen at the onset of exercise, and the constancy of muscle $\dot{V}O_2$ kinetics determined by the mitochondrial content of the contracting muscle group.

The muscle oxygen uptake at any time in point ($\dot{V}O_{2m}(t)$) is given by the instantaneous product of the muscle blood flow ($q_m(t)$) and the corresponding arterio–venous oxygen differences ($\Delta m(t)$). Assuming that the time course of q_m and Δm are both monoexponential:

$$q_m(t) = q_m(s)(1 - \exp(-t/\tau q)) \quad (4)$$

$$\Delta m(t) = \Delta m(s)(1 - \exp(-t/\tau\Delta)) \quad (5)$$

where the suffix s denotes the steady-state values and the τ values are indicated by τq and $\tau\Delta$.

It can easily be shown that the time course of the product of Eqs. 4 and 5 (i.e. of the $\dot{V}O_{2m}(t)$) must be faster that each of them: since the following applies:

$$1/\tau q + 1/\tau\Delta = 1/\Delta\dot{V}O_2 \quad (6)$$

where $\Delta\dot{V}O_2$ is the τ of the $\dot{V}O_2$ response at the muscle level. In these experiments, the experimental quantity of mOxy is in fact proportional to the quantity indicated by Δm in the above equations:

$$\Delta m = [\mathrm{OxyHb}]k \quad (7)$$

where [OxyHb] is the oxyhemoglobin concentration in blood (assumed to be constant) and k is the oxygen carrying capacity of the Hb (1.34 ml g^{-1}). It is also assumed that the contribution of oxygen bound to myoglobin is negligible.

Therefore, we can conclude from the present data that the kinetics of $\dot{V}O_{2m}$ must be described by $\tau O_2 \leq 13$ s, i.e. smaller than the average value reported in Table 2 for the MRT of mOxy. This is faster than generally accepted, even though some recent data of Cautero et al. (2002) tend to sugest similarly fast $\dot{V}O_2$ on responses at the muscle level.

In conclusion, we found that the mOxy kinetics at work onset are constant over a wide range of work loads. Although the factors limiting mOxy kinetics are still largely unknown, the results of this study are consistent with the hypothesis that during rest-to-exercise transitions in humans, the kinetics of muscle $\dot{V}O_2$ are dictated by metabolic factors.

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