Relationship between Oxygenation in Inactive Biceps Brachii Muscle and Hyperventilation during Leg Cycling

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Received October 26, 2006
Accepted January 19, 2006
On-line available February 23, 2006

Summary
Inactive forearm muscle oxygenation has been reported to begin decreasing from the respiratory compensation point (RCP) during ramp leg cycling. From the RCP, hyperventilation occurs with a decrease in arterial CO₂ pressure (PaCO₂). The aim of this study was to determine which of these two factors, hyperventilation or decrease in PaCO₂, is related to a decrease in inactive biceps brachii muscle oxygenation during leg cycling. Each subject (n = 7) performed a 6-min two-step leg cycling. The exercise intensity in the first step (3 min) was halfway between the ventilatory threshold and RCP (170±21 watts), while that in the second step (3 min) was halfway between the RCP and peak oxygen uptake (240±28 watts). The amount of hyperventilation and PaCO₂ were calculated from gas parameters. The average cross correlation function in seven subjects between inactive muscle oxygenation and amount of hyperventilation showed a negative peak at the time shift of zero (r = -0.72, p<0.001), while that between inactive muscle oxygenation and calculated PaCO₂ showed no peak near the time shift of zero. Thus, we concluded that decrease in oxygenation in inactive arm muscle is closely coupled with increase in the amount of hyperventilation.

Key words
Hyperventilation • Inactive muscle • Oxygen supply • Arterial carbon dioxide pressure

Introduction
We have recently examined oxygenation kinetics in inactive forearm muscle during ramp leg cycling (Ogata et al. 2004). Change in oxygenation in inactive muscle was used as an index of change in oxygen supply, since oxygenation is influenced by oxygen consumption and oxygen supply and since oxygen consumption is assumed to be almost constant in inactive muscle (Ogata et al. 2002, Yano et al. 2005). We found that oxygenation in inactive forearm muscle began to decrease from the respiratory compensation point (RCP) during ramp leg cycling. From the RCP, the rate of increase in minute ventilation (VE) against power output begins to rise abruptly. The hyperventilation is accompanied by a decrease in arterial CO₂ pressure. Hypocapnia is known to decrease cerebral oxygenation (Grote et al. 1981, Hampson and Piantadosi 1990), but the results for
skeletal muscle are contradictory (Gustafsson et al. 1993, Hampson and Piantadosi 1990). Thus, we hypothesized that hyperventilation per se may be related to a decrease in oxygenation in inactive arm muscle during leg cycling. The aim of the present study was to determine which factor, hyperventilation or decrease in PaCO₂, is related to a decrease in oxygenation in inactive biceps brachii muscle during leg cycling. In the present study, the amount of hyperventilation was extracted from the actual VE using the method of Koyal et al. (1976), and the change in PaCO₂ was predicted from the change in end-tidal CO₂ pressure using the method of Jones et al. (1979).

Methods

Subjects

Seven healthy male subjects participated in this study. They had no cardiovascular risk factors, were not taking any medication, and were not engaged in any formal exercise-training program. Mean values (± SD) of age, height and weight of the subjects were 24±2 years, 175±7 cm and 70±7 kg, respectively. Voluntary consent for the participation in this study was obtained from all subjects after informing them of the purpose of the experiment, the procedure, and possible risks. This study was approved by the local ethics committee.

Experimental protocol

Ramp leg cycling was performed using a bicycle ergometer (Ergometer 232CXL, Combi, Tokyo, Japan) in an upright position to determine exercise intensities at the ventilatory threshold (VT), RCP and peak oxygen uptake (VO₂peak). Before the experiment, each subject sat on the saddle with his toes on the pedals. Then the seat height was adjusted so that there was a slight bend in the knee joint when the foot pedal was at its lowest point. At the start of the experiment, subjects rested for 5 min and then the work load was increased by 20 Watts every minute until exhaustion. VO₂peak was defined as the peak value obtained in this test.

On separate days after completion of ramp exercise, each subject performed two-step incremental leg cycling (LCinc). Before the exercise, each subject rested for 5 min and then performed a 6-min exercise. After the exercise, each subject recovered for 5 min. The intensity of exercise in the first step (3 min) was midway between VT and RCP, while that in the second step (3 min) was halfway between RCP and VO₂peak. The reason for using this protocol is as follows. Hyperventilation occurs during exercise above the VT. The level of hyperventilation is smaller than that observed from the RCP. This characteristic is useful if the magnitude of decrease in muscle oxygenation is compared to the magnitude of increase in the amount of hyperventilation. Each subject also performed two-step decremental exercise (LCdec). The intensity of exercise in the first step (3 min) was halfway between RCP and VO₂peak, while that in the second step (3 min) was halfway between VT and RCP. In this exercise, the pattern of change in hyperventilation dissociates completely from that in predicted PaCO₂ as shown in Figure 1. This characteristic is useful to examine the relationship to change in inactive muscle oxygenation. These two experiments were performed on different days and in random order.

All exercises, including ramp and two-step exercises, were performed at 60 rpm. The subjects were asked to keep their arms resting as much as possible on a table adjusted to the height of heart level throughout the rest, exercise and recovery periods.

Measurements

Change in muscle oxygenation was determined using a near-infrared spectrometer (NIRS, HEO200N, Omron, Tokyo, Japan) as described in detail elsewhere (Ogata et al. 2004). It is known that both hemoglobin (Hb) and myoglobin (Mb) absorb NIRS radiation. There are conflicting data regarding the influence of myoglobin on the NIRS signal (Mancini et al. 1994, Tran et al. 1999). It is considered that NIRS oxygenation values represent volume-averaged values in the portion of tissue under consideration, i.e. coming from Hb and Mb (Grassi et al. 2003). NIRS provides separate measures of changes in concentrations of oxygenated Hb+Mb (oxyHb/Mb) and deoxygenated Hb+Mb (deoxyHb/Mb). In the present study, oxyHb/Mb was used as a measure of muscle oxygenation.

Based on the method used in previous investigations (Hamaoka et al. 1996, Higuchi et al. 2002), change in oxyHb/Mb was normalized using the arterial occlusion method, and the relative change in oxyHb/Mb (%) was estimated in the present study. The minimum oxyHb/Mb level during arterial occlusion was regarded as 0 %, and the oxyHb/Mb level at rest was regarded as 100 %. The resting level was regarded as an average of data between the first and the fourth minutes during a 5-min resting period.

In the two-step exercise tests, the NIRS probe
Fig. 1. Changes in oxygenated hemoglobin/myoglobin concentration in inactive biceps brachii muscle (top), minute ventilation and amount of hyperventilation (middle), and predicted arterial CO₂ pressure (bottom) during two-step incremental and decremental exercises (left and right, respectively). In the middle panels, an open circle (○) denotes minute ventilation, while a filled circle (●) denotes amount of hyperventilation. The amount of hyperventilation was defined as the difference between actual minute ventilation (VE) and VE estimated using the oxygen uptake-VE relationship below the ventilatory threshold during ramp leg cycling. PaCO₂pre was calculated from end-tidal CO₂ pressure. During two-step incremental exercise, the intensity of exercise in the first step (3 min) was halfway between the ventilatory threshold (VT) and respiratory compensation point (RCP) recorded during ramp leg cycling, while that in the second step (3 min) was halfway between the RCP and maximal oxygen uptake (VO₂peak). During two-step decremental exercise, on the other hand, the intensity of exercise in the first step (3 min) was halfway between the RCP and VO₂peak, while that in the second step (3 min) was halfway between the VT and RCP. Values are means ± S.E.M. (n = 7).
was fixed over the belly of the biceps brachii muscle of the right forearm of each subject and NIRS signals were measured during rest, exercise, and recovery periods with a sampling time of 5 s. The averages of these data were calculated for each 15-s interval and these averages were used for statistical analysis.

Data on VE, oxygen uptake (VO2), carbon dioxide (VCO2), end-tidal O2 pressure (PETO2) and end-tidal CO2 pressure (PETCO2) were obtained breath-by-breath using a respiratory gas analyzer (AE-280S, Minato Medical Science, Osaka, Japan). VE was measured by a hot-wire flow meter, and the flow meter was calibrated with a syringe of known volume (2.0 l). O2 and CO2 concentrations were measured by a zirconium sensor and infrared absorption analyzer, respectively. The gas analyzer was calibrated by known standard gas (O2 15.17 %, CO2 4.92 %). Heart rate (HR) was recorded using a heart rate monitor installed in the respiratory gas analyzer. These data were measured continuously during rest, exercise, and recovery periods. The averages of these data were calculated for each 15-s interval and these averages were used for statistical analysis.

Blood samples (each 25 μl) were taken from a fingertip and immediately analyzed for blood lactate concentration ([La–]) using a blood lactate analyzer (1500 Sport, YSI, Ohio, USA). The analyzer was calibrated by known standard solution (5 mmol). In the two-step exercise tests, blood samples were collected at rest and at the 3rd and 6th min of exercise.

Data analysis

VT was determined using the following criteria: i) an increase in VE relative to VO2, ii) an increase in VCO2 relative to VO2, iii) an increase in PETO2 without a decrease in PETCO2, and biv) an increase in VE/VO2 without an increase in VE/VCO2 (Scheuermann and Kowalchuk 1998). The respiratory compensation point (RCP) was determined as the point at which VE/VCO2 began to increase and at which PETCO2 began to decrease following the isocapnic buffering phase (Oshima et al. 1997, Scheuermann and Kowalchuk 1998, Takano 2000).

Based on the method of Koyal et al. (1976), the amount of hyperventilation (VEhyper) during each two-step exercise was calculated in the following manner. A linear regression line of VE against VO2 was made using data below the VT recorded during ramp leg cycling. VO2 obtained during each two-step exercise was substituted into the expression of the linear regression line and predicted VE was calculated. The difference between predicted VE and actual VE obtained during two-step exercise was defined as VEhyper.

Arterial CO2 pressure (PaCO2) was predicted from PETCO2 using the following equation (Jones et al. 1979):

\[
\text{predicted PaCO}_2 (\text{PaCO}_2_{\text{pre}}) = 5.5 + 0.90 \cdot \text{PETCO}_2 - 0.0021 \cdot \text{tidal volume}, \text{where PaCO}_2_{\text{pre}} \text{ and PETCO}_2 \text{ are in Torr and tidal volume is in ml.}
\]

Cross-correlation was applied to the following time series: 1) oxyHb/Mb and VEhyper in LCinc; 2) oxyHb/Mb and PaCO2pre in LCinc; 3) oxyHb/Mb and VEhyper in LCdec; and 4) oxyHb/Mb and PaCO2pre in LCdec. To evaluate the cross correlation coefficient and time shift between two time series, the cross correlation function (CCF) was applied. We determined the negative peak of the CCF between oxyHb/Mb and VEhyper and the positive peak of the CCF between oxyHb/Mb and PaCO2pre. Time shifts from zero to the time point at which the peaks were observed were also determined.

Paired student’s t-test was used for comparison of the values at the 3rd and the 6th minutes of exercise and for comparison of the values at the 3rd min of LCinc and the 6th min of LCdec. A value of p<0.05 was regarded as statistically significant. All data are presented as means ± SE.

Results

The highest values of work rate at exhaustion and peak HR obtained during ramp leg cycling were 270±12 Watts and 175±2 bpm, respectively. Table 1 shows the work rate, cardiorespiratory data measured at rest, VT, RCP and VO2 peak. The values of VO2 at the VT and RCP were 50±2 and 77±2 % of VO2 peak, respectively.

The work rates halfway between VT and RCP and halfway between RCP and VO2 peak were 170±7 and 240±10 Watts, respectively. These values corresponded to 63±1 and 89±1 % of peak work rate, respectively. Table 2 shows cardiorespiratory, [La–] and oxyHb/Mb data recorded at rest, at the 3rd and the 6th min of exercise, and at the 5th min of recovery. Values of VO2 recorded at the 3rd and 6th min of LCinc corresponded to 76±2 and 103±1 % of VO2 peak, respectively, while those recorded at the 3rd and the 6th minutes of LCdec corresponded to 98±2 and 93±3 % of VO2peak, respectively. The value of VO2 at the 6th min of LCinc exceeded the value of VO2peak recorded during ramp leg cycling. The reason for this is that VO2peak recorded
Figures 1A and 1B show average changes in oxyHb/Mb in LCinc and LCdec, respectively. oxyHb/Mb began to decrease about 30 s after the onset of exercise in both LCinc and LCdec. In LCinc, after the onset of the second step, oxyHb/Mb also began to decrease with a time delay of about 30 s. The value of oxyHb/Mb recorded at the 6th min was significantly lower than that recorded at the 3rd min (Table 2). In LCdec, after the onset of the second step, oxyHb/Mb increased gradually with time. However, the value of oxyHb/Mb recorded at the 6th min was not significantly different from that recorded at the 3rd min (Table 2). Compared to the value at the 3rd min of LCinc, the value of oxyHb/Mb recorded at the 6th min of LCdec was significantly lower despite the fact that the exercise intensities were the same (Table 2).

Figures 1C and 1D show average changes in \( V_{E,hyper} \) in LCinc and LCdec, respectively. \( V_{E,hyper} \) began to increase about 45 s after the onset of exercise in both LCinc and LCdec. In LCinc, \( V_{E,hyper} \) increased more after the onset of the second step. The value of \( V_{E,hyper} \) recorded at the 6th min was significantly higher than that recorded at the 3rd min (Table 2). In LCdec, after the onset of the second step, \( V_{E,hyper} \) decreased gradually with time. However, the value of \( V_{E,hyper} \) recorded at the 6th min was significantly different from that recorded at the 3rd min (Table 2).
was not significantly different from that recorded at the 3rd min (Table 2). Compared to the value at the 3rd min of LCinc, the value of Vehyper recorded at the 6th min of LCdec was significantly higher despite the fact that the exercise intensities were the same (Table 2).

Figures 1E and 1F show average changes in PaCO2pre in LC inc and LC dec, respectively. In contrast to the case of oxyHb/Mb and Vehyper, the patterns of changes in PaCO2pre were similar in LCinc and LCdec. Briefly, PaCO2pre began to increase from the onset of exercise up to about the 1st min of exercise. Thereafter, PaCO2pre decreased gradually until the end of exercise.

Table 2 shows the values of [La–]. In both LCinc and LCdec, the value of [La–] recorded at the 6th min were significantly higher than those recorded at the 3rd min. Compared to the value at the 3rd min of LCinc, the value of [La–] recorded at the 6th min of LCdec was significantly higher despite the fact that the exercise intensities were the same.

Figures 2A and 2B show CCF between oxyHb/Mb and Vehyper in each two-step exercise test. The data are averages for seven subjects. In both LCinc and LCdec, the CCF between oxyHb/Mb and Vehyper showed a clear negative peak at the time shift of zero. The ranges of the time shift in seven subjects were between –60 and 30 s in LCinc and between –90 and 30 s in LCdec. Two subjects showed a negative peak at the time shift of zero in both LCinc and LCdec. All peaks were statistically significant.

Figures 2C and 2D show CCF between oxyHb/Mb and PaCO2pre in each two-step exercise test. In both LCinc and LCdec, no positive peak was observed near the time shift of zero.

Discussion

The main finding from this study is that the decrease in oxyHb/Mb in inactive biceps muscle is closely coupled with the increase in Vehyper but not with the decrease in PaCO2pre during leg cycling. Since PaCO2pre was used as an estimate of actual arterial CO2 pressure, it might be desirable to detect a possible
difference between changes in oxyHb/Mb and PaCO$_{2\text{pree}}$ for determination of the difference between changes in oxyHb/Mb and actual PaCO$_2$. Indeed, we obtained such an evidence: changes in PaCO$_{2\text{pree}}$ in LC$_{\text{inc}}$ and LC$_{\text{dec}}$ were very similar, whereas changes in oxyHb/Mb were completely different in the two exercise modes. Thus, we can safely state that change in oxyHb/Mb in inactive muscle is dissociated from that in actual PaCO$_2$.

The oxyHb/Mb in inactive muscle began to decrease 30 s after the onset of exercise in both LC$_{\text{inc}}$ and LC$_{\text{dec}}$. A similar result was found in our previous study (Ogata et al. 2002) in which we showed that a decrease in oxygenation in inactive vastus lateralis muscle during arm cranking started more than 1 min after the onset of exercise. We attributed the decrease in oxygenation to the decrease in blood flow. In the present study, in LC$_{\text{inc}}$, the magnitude of decrease in oxyHb/Mb became larger after the onset of the second step at a higher intensity. Taylor et al. (1992) observed that the magnitude of decrease in blood flow in the inactive forearm during leg cycling became larger with the increase in exercise intensity. Thus, a decrease in oxygen supply secondary to blood flow reduction caused the decrease in oxyHb/Mb in inactive muscle in the present study.

Sheel et al. (2002) reported that inactive leg blood flow at rest was reduced when the fatiguing inspiratory muscle work was loaded via voluntary increase in inspiratory effort against resistance. Sheel et al. (2002) also suggested that the sympathetic vasoconstriction was responsible for the decrease in the inactive leg blood flow during the inspiratory work because St Croix et al. (2000) found that the sympathetic nerve activity was increased by the same inspiratory muscle work. Thus, one might expect that the relationship between changes in oxyHb/Mb in inactive muscle and VE$_{\text{hyper}}$ becomes manifested because fatiguing respiratory muscle work associated with hyperventilation causes a decrease in blood flow in inactive arm muscle. However, fatigue of the diaphragm occurs during whole body endurance exercise in excess of 85 % of maximal oxygen uptake (Johnson et al. 1993). This exercise intensity is above exercise intensity at which the decrease in oxyHb/Mb was observed in the present study (76 % VO$_{2\text{peak}}$ in the first step of LC$_{\text{inc}}$).

Although we have no direct evidence for the association of the decrease in oxyHb/Mb with the increase in VE$_{\text{hyper}}$, we can propose the following working hypothesis. In the present study, the level of [La$^+$] during the second step of LC$_{\text{dec}}$ was larger than that during the first step of LC$_{\text{inc}}$ despite the fact that work loads were the same. This larger increase in [La$^+$] was accompanied by a larger decrease in oxyHb/Mb. In our previous study (Ogata et al. 2002), it was demonstrated that heavy arm cranking reduced oxygenation in inactive vastus lateralis muscle, whereas moderate arm cranking had no effect on oxygenation in inactive muscle. In addition, the decrease in inactive muscle oxygenation during heavy arm cranking started with a time delay of more than 1 min. These results suggest the decrease in oxygenation in inactive muscle is coupled with accumulation of metabolites. The accumulation of metabolites results in metabolic acidosis. The acidosis is known to cause hyperventilation via autonomic reflexes evoked by stimulation on muscle metaboreceptors (Oelberg et al. 1998, Systrom et al. 2001) or carotid bodies (Wasserman et al. 1975). Thus, the decrease in inactive muscle oxygenation might be related to hyperventilation during exercise.

In our previous study (Ogata et al. 2004), we examined the kinetics of oxygenation in inactive forearm muscle during ramp leg cycling and could not find an apparent decrease in inactive muscle oxygenation from the VT at which hyperventilation occurs. In that study, we used ramp leg cycling at an increasing rate of 10 watts·min$^{-1}$ in power output. According to the results obtained by Scheuermann and Kowalchuk (1998), the rate of increase in VE$_{\text{hyper}}$ per minute is lower during slow ramp exercise (8 watts·min$^{-1}$) than during fast ramp exercise (65 watts·min$^{-1}$). Thus, the decrease in inactive muscle oxygenation from the VT might be obscured during slow ramp exercise.

An increase in muscle oxygen consumption has been shown to be involved in a decrease of muscle oxygenation (Hamaoka et al. 1996). In the present study, however, the subjects were asked to keep their arms resting as much as possible on a table adjusted just below the height of heart level throughout the rest and exercise periods. Thus, the effect of an increase in muscle oxygen consumption on decrease in oxyHb/Mb would have been minimal.

In conclusion, the magnitude of decrease in oxygen supply to inactive muscle is closely coupled to the magnitude of increase in the amount of hyperventilation during exercise. This association may arise because accumulated metabolites have an effect concurrently on both oxygen supply to inactive muscle and hyperventilation.
References


**Reprint requests**
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