

Ryotaro Kime · Takafumi Hamaoka · Takayuki Sako  
Motohide Murakami · Toshiyuki Homma  
Toshihito Katsumura · Britton Chance

## Delayed reoxygenation after maximal isometric handgrip exercise in high oxidative capacity muscle

Accepted: 18 October 2002 / Published online: 24 December 2002  
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**Abstract** We hypothesized that after maximal short-term isometric exercise, when  $O_2$  demand is still high and  $O_2$  supply is not fully activated, higher oxidative capacity muscle may exhibit slower muscle reoxygenation after the exercise than low oxidative capacity muscle. Seven healthy male subjects performed a maximal voluntary isometric handgrip exercise for 10 s. The reoxygenation rate after the exercise (Reoxy-rate) in the finger flexor muscle was determined by near infrared continuous wave spectroscopy (NIRcws) while phosphocreatine (PCr) was measured simultaneously by  $^{31}P$  magnetic resonance spectroscopy. Muscle oxygen consumption (muscle  $\dot{V}O_2$ ) and muscle oxidative capacity were evaluated using the rate of PCr resynthesis post-exercise. The forearm blood flow (FBF) index at the end of exercise was measured using NIRcws. There was a significant positive correlation between the Reoxy-rate, which ranged between  $0.53\% s^{-1}$  and  $12.47\% s^{-1}$ , and the time constant for PCr resynthesis, which ranged between 17.8 s and 38.3 s ( $r^2=0.939$ ,  $P<0.001$ ). At the end of the exercise, muscle  $\dot{V}O_2$  exceeded the resting level by approximately 25-fold, while the FBF index exceeded the resting level by only 3-fold on average. The Reoxy-rate closely correlated with muscle  $\dot{V}O_2$  ( $r^2=0.727$ ,  $P<0.05$ ), but not with the FBF index. Also, the estimated  $O_2$  balance (muscle  $\dot{V}O_2$  index/FBF index) was negatively correlated with the Reoxy-rate ( $r^2=0.820$ ,

$P<0.001$ ). These results support our hypothesis that higher oxidative capacity muscle shows slower muscle reoxygenation after maximal short-term isometric exercise because the Reoxy-rate after this type of exercise may be influenced more by muscle  $\dot{V}O_2$  than by  $O_2$  supply.

**Keywords** Reoxygenation rate · Muscle oxidative capacity · Near infrared spectroscopy · Phosphorus magnetic resonance spectroscopy · Isometric exercise

### Introduction

One of the methods for assessing muscle oxidative capacity in vivo includes muscle biopsy (Harris et al. 1974; Esbjornsson et al. 1993; Chilibeck et al. 1998; Mizuno et al. 1999). Although muscle biopsy may be useful and regarded as the gold standard, it is cumbersome and inconvenient to apply in many physiological and clinical conditions because of its invasive nature. During both steady state and non-steady-state conditions, phosphorus magnetic resonance spectroscopy ( $^{31}P$ -MRS) can provide a non-invasive and repeated method of measuring muscle energy metabolism. The MRS device, however, is rather expensive and requires careful maintenance for precise measurements. Furthermore, this methodology has its own limitations in practical use.

During the 1990s, near-infrared spectroscopy (NIRS) was used in studying of exercising skeletal muscle in humans (Chance et al. 1992). Over the past several years, many more groups have applied this technique (McCully et al. 1994; Hamaoka et al. 1998; Higuchi et al. 2002). The near-infrared continuous wave spectroscopy (NIRcws) methodology is a useful tool because it is both non-invasive and inexpensive. The parameters commonly measured by NIRcws are oxygenated hemoglobin and/or myoglobin (Oxy-Hb/Mb), deoxygenated hemoglobin and/or myoglobin (Deoxy-Hb/Mb), and total hemoglobin ( $Hb_{tot}$ ). The NIRcws technique, however, has its

R. Kime (✉) · B. Chance  
Department of Biochemistry and Biophysics,  
250 Anatomy–Chemistry Building,  
University of Pennsylvania,  
37th and Hamilton Walk,  
Philadelphia, PA 19104-6059, USA  
E-mail: ZXC06104@nifty.com  
Tel.: +1-215-8984342  
Fax: +1-215-8981806

R. Kime · T. Hamaoka · T. Sako · M. Murakami · T. Homma ·  
T. Katsumura  
Department of Preventive Medicine and Public Health,  
Tokyo Medical University,  
6-1-1 Shinjuku-ku,  
Tokyo, 160-8402, Japan

own difficulties in measuring energy metabolism during exercise. This is because absolute concentration changes of Hb/Mb cannot be determined due to unquantifiable biophysical quantities (i.e., the optical path length). More recently, some studies have reported that different NIRS technologies (time-resolved spectroscopy, NIR<sub>TRS</sub>; spatially resolved spectroscopy, NIR<sub>SRS</sub>) can monitor absolute tissue saturation. However, it is difficult to apply NIR<sub>TRS</sub> during exercise because of its low time resolution (Patterson et al. 1989; Hamaoka et al. 2000a). On the other hand, NIR<sub>SRS</sub> cannot accurately calculate absolute saturation because it uses optical signals of different but close measurement areas (Quaresima et al. 2001).

Although NIRcws has a higher time resolution (up to 50 Hz) than NIR<sub>TRS</sub>, NIRcws can only measure the relative changes of Oxy-Hb/Mb and Deoxy-Hb/Mb as the balance between muscle oxygen consumption (muscle  $\dot{V}O_2$ ) and O<sub>2</sub> supply. Because of this limitation, several studies have investigated the relationship between the post-exercise recovery rate of Oxy-Hb/Mb, and muscle oxidative capacity (Chance et al. 1992; McCully et al. 1994; Hamaoka et al. 1998; Mizuno et al. 1999). The results, however, are not consistent. Some investigators reported that the muscle reoxygenation rate after isometric plantar flexion exercise does not correlate with muscle oxidative capacity (Hamaoka et al. 1998; Mizuno et al. 1999). In contrast, however, other studies have shown fast Hb/Mb recovery in high oxidative capacity muscle (HOCmuscle) (Chance et al. 1992; McCully et al. 1994). In particular, McCully et al. (1994) reported that phosphocreatine (PCr) recovery and Oxy-Hb/Mb showed similar behavior after dynamic plantar flexion exercise with muscle pH being 7.0. These divergent results, which appear to be due to slight differences in the exercise paradigm, suggest that post-exercise Oxy-Hb/Mb may be influenced more by O<sub>2</sub> supply during dynamic exercise than during isometric exercise.

Again, because the Hb/Mb reoxygenation rate (Reoxy-rate) reflects the balance between muscle  $\dot{V}O_2$  and O<sub>2</sub> supply in localized muscle, the Reoxy-rate determined by NIRcws can be interpreted in various ways. When the O<sub>2</sub> supply is limited, post-exercise Reoxy-rate may be more influenced by muscle  $\dot{V}O_2$ . Blood flow after static muscular exercise is lower than after dynamic exercise (Huonker et al. 1996). Also, blood flow is the lowest at the beginning of exercise and increases exponentially during exercise (Hughson et al. 1996; Saltin et al. 1998). We hypothesized that during post-maximal short-term handgrip isometric exercise, when O<sub>2</sub> demand is high and O<sub>2</sub> supply is not sufficient, HOCmuscle would show a greater increase in muscle  $\dot{V}O_2$  than low oxidative capacity muscle (LOCmuscle) during the recovery phase. In contrast, the O<sub>2</sub> supply would not increase as much as muscle  $\dot{V}O_2$  after the exercise. Therefore, it is expected that after a short duration, maximal isometric exercise, HOCmuscle would exhibit slower muscle oxygenation recovery. The primary goal of this study was to test the hypothesis that higher

oxidative capacity muscle exhibits a slower muscle oxygenation recovery after short-term maximal isometric exercise. We further evaluated muscle  $\dot{V}O_2$  and O<sub>2</sub> supply separately to validate the O<sub>2</sub> balance determined by NIRcws (Reoxy-rate) after this type of exercise.

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## Methods

### Subjects

Seven healthy male volunteers [five moderately active, one well-trained triathlete, and one former Japanese national windsurfing champion, mean age (SD): 29 (3) years] participated in this study. All subjects were briefed about the experimental protocol, and informed consent was obtained before the test.

### Experimental design

The muscle oxygenation level was measured by NIRcws while the PCr concentration was measured simultaneously by <sup>31</sup>P-MRS (Sako et al. 2001). The rate of Oxy-Hb/Mb recovery immediately after exercise up until 6 s after exercise was defined as the Reoxy-rate. Subjects performed the same experiment twice to examine the Reoxy-rate, the time constant for PCr recovery (PCr<sub>Tc</sub>) and muscle  $\dot{V}O_2$  at the end of exercise (muscle  $\dot{V}O_{2EX}$ ) (experiment 1) and the forearm blood flow (FBF) index (experiment 2). These were measured once using <sup>31</sup>P-MRS and NIRcws (experiment 1) and once using NIRcws only (experiment 2) (Fig. 1). This dual measurement was performed because the FBF index from the NIR signal required venous occlusion at the end of exercise. To ensure familiarity with the exercise modality, each subject engaged in two to three bouts of isometric handgrip practice before the start of the exercise protocol.

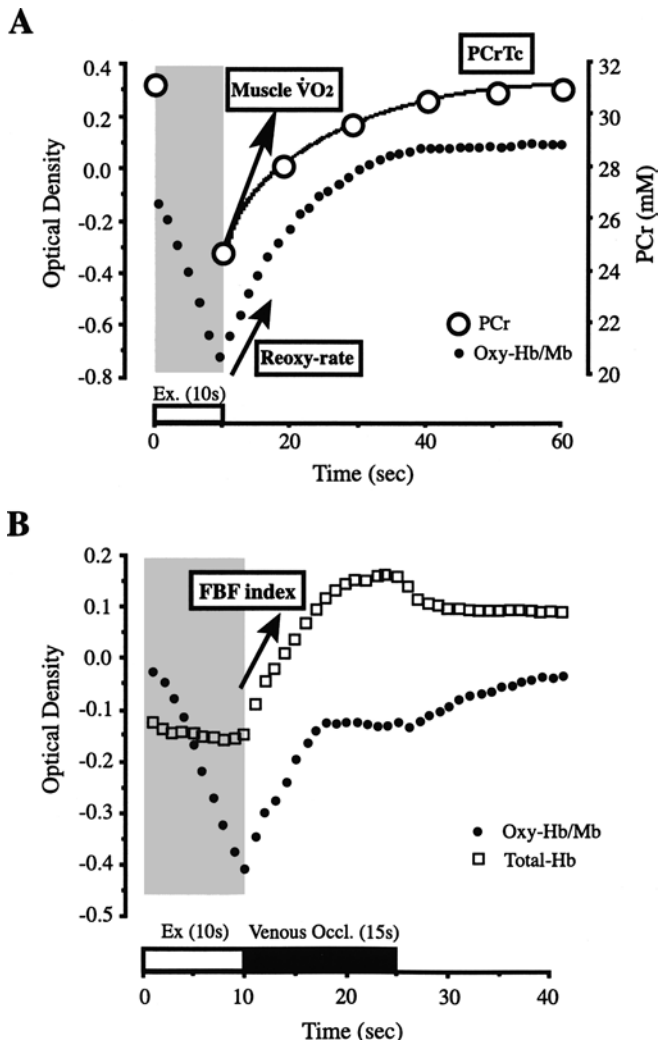
### Experimental protocol

Each subject sat on an adjusted chair that allowed the arm to extend to the level of the heart. The forearm was secured to a platform that contained a 3.0 cm diameter circular surface coil for <sup>31</sup>P-MRS as well as a light source and optical detector with a distance of 3.0 cm for NIRcws (Sako et al. 2001). The <sup>31</sup>P-MRS spectra measurement area was defined by the coil circumference and penetrated to a depth equal to the radius of the coil (McCully et al. 1989; Richardson et al. 1995). The mean penetration depth of near infrared light into the tissue was equal to approximately half the distance between the input and output fibers (Chance et al. 1992). Therefore, both devices could simultaneously take measurements at nearly identical sites. The measurement site was the right finger flexor muscle, the most active site in handgrip contraction. The subjects performed a maximal isometric handgrip exercise for 10 s and the arm was completely relaxed immediately after exercise.

### Near-infrared continuous wave spectroscopy

During and after exercise, the oxygenation level in the finger flexor muscles was measured within the magnetic fields using NIRcws (OM100A, Shimadzu, Kyoto, Japan). The basic operation of this NIRcws device has been extensively explained in previously published papers (Chance et al. 1992; Hamaoka et al. 1996; Homma et al. 1996a).

It is well known that the level of oxygenation of Hb/Mb alters the absorption of the light in muscle tissue. For example, as Hb/Mb is oxygenated, the absorbance at 780 nm decreases and the absorbance at 830 nm increases, providing a different signal.



**Fig. 1** **A** The Reoxy-rate,  $PCr_{TC}$ , and muscle  $\dot{V}O_{2EX}$  values (experiment 1). Muscle  $\dot{V}O_{2EX}$  was calculated by differentiating the monoexponential curve. **B** The FBF index value after exercise (experiment 2). *Reoxy-rate* Reoxygenation rate, *PCr<sub>TC</sub>* time constant for phosphocreatine (PCr) resynthesis, *muscle  $\dot{V}O_{2EX}$*  muscle oxygen consumption at the end of exercise, *FBF* forearm blood flow, *Ex* maximal isometric handgrip exercise, *Venous Occl* venous occlusion

Accordingly, the detectable light intensities of the detector change due to the level of Hb/Mb deoxygenation. Both Hb/Mb absorb light equally at the wavelength employed. However, it is reported that Mb concentration is no greater than 25% (Chance et al. 1992) or 20% (Mancini et al. 1994). Therefore, we can conclude that the signals are derived mainly from Hb.

In this study, three-wavelength optical fibers, with wavelengths of 780 nm, 805 nm and 830 nm, were used as the light source. Changes in Oxy-Hb/Mb and  $Hb_{tot}$  were calculated with the least-squares method using data from the changes in light absorbance at different wavelength (Homma et al. 1996a). The reliability of this algorithm was previously confirmed in vivo (Homma et al. 1996a; Sako et al. 2001). However, there are some disadvantages in using the NIRcws technique to evaluate muscle energy metabolism during exercise. Most notably, measurement sensitivity is impaired by the influence of adipose tissue (Homma et al. 1996b). The thickness of subcutaneous adipose tissue varies greatly in humans and the optical densities of Oxy-Hb/Mb and  $Hb_{tot}$  cannot be compared between individuals (Quaresima and Ferrari 2002). Therefore, to evaluate differences in muscle oxygenation between each subject,

arterial occlusion was used (Hamaoka et al. 1996; Sako et al. 2001); this technique involves interrupting arterial blood flow by placing a pneumatic tourniquet on the upper arm at a pressure of 280 mmHg. The pre-occluded resting level of Oxy-Hb/Mb was defined as 100% and the minimum value during ischemia as 0%. The muscle oxygenation level during and after exercise was expressed using the overall change from the resting pre-occluded to the minimum oxygenation level during arterial occlusion.

Due to the fact that the NIRcws machine's time resolution (time resolution = 1.5 s), which was simultaneously measured with  $^{31}P$ -MRS, does not accurately estimate the FBF index during venous occlusion, another NIRcws device (HEO-210, OMRON, Kyoto, Japan; time resolution = 0.1 s) was used out of the magnetic field. Two-wavelength light-emitting diodes, with wavelengths of 760 nm and 840 nm, were used as the light source. The reliability of this method of calculating signals was previously confirmed with in vitro (Shiga et al. 1997) and in vivo experiments (Hamaoka et al. 1996; Shiga et al. 1997; Hamaoka et al. 1998). The FBF index as determined by venous occlusion is fully described in a previously published paper (Homma et al. 1996a; Van Beekvelt et al. 2001). The resting FBF index was estimated by measuring the peak rate of increase in  $Hb_{tot}$  during venous occlusion for 15 s. This procedure was repeated five times every 1 min before the principal exercise; then the mean value was used as the resting FBF index. The FBF index at the end of exercise was measured by venous occlusion immediately after exercise. Before the experiment, the optimal cuff pressure was determined for each subject to obtain the greatest increase in the  $Hb_{tot}$  rate during venous occlusion. On average, the cuff pressure used was 70.0 (0.35) [mean (SD)] mmHg.

### $^{31}P$ Phosphorus magnetic resonance spectroscopy

Phosphorus metabolites during rest, exercise, and recovery were monitored by  $^{31}P$ -MRS (Otsuka Electronics Inc.) with a 2.0-T superconducting 26-cm-bore magnet. A 3.0 cm diameter circular double-turn surface coil was attached to a cradle located at the mid-forearm, centered mainly over the flexor digitorum superficialis muscle; it was tuned to both the  $^{31}P$  and  $^1H$  frequencies (34.6 MHz and 85.4 MHz, respectively). The homogeneity of the magnetic field was adjusted to <0.28 parts per million on the proton signal from water.  $^{31}P$  spectra were obtained using a 60- $\mu$ s pulse width to optimize signal intensity. The pulse repetition time was 2 s. Five pulses were averaged during to obtain a free induction decay (FID), so each spectrum was obtained every 10 s.

The FIDs were obtained and Fourier transformed into spectra; the peak areas were fitted by a non-linear, curve-fitting method to calculate the areas under the  $\beta$ -ATP, PCr, and  $P_i$  peaks in the frequency domain that minimizes the sum of squares between the spectral resonances. To acquire the saturation factors for each phosphate compound, a ratio was calculated between each area obtained at 20-s repetition time (fully relaxed spectra) and those obtained at 2-s pulse intervals. Then, PCr and  $P_i$  peak areas were quantified from the ratio of PCr and  $P_i$  areas to  $\beta$ -ATP area, with corrections made for the saturation factor. Because there were no data available for a direct chemical analysis of the forearm muscles, the ATP concentration reported from the muscle biopsies of the human vastus lateralis ( $[ATP]=8.2$  mM) was used for PCr and  $P_i$  quantification (Harris et al. 1974). The intracellular pH was calculated from the median chemical shift between the  $P_i$  and PCr peaks (Kushmerick and Meyer 1985).

### Data analysis

The area of the PCr peaks during recovery was integrated and fitted to a single-exponential curve, and the time constant for PCr resynthesis ( $PCr_{TC}$ ) was determined using the following equation:

$$PCr = PCr_0 + \Delta PCr(1 - e^{-kt})$$

where PCr is the PCr concentration at time  $t$  during recovery,  $PCr_0$  is the PCr concentration at the end of exercise,  $\Delta PCr$  is the difference between final and initial PCr,  $t$  is the time,  $k$  is the rate constant of the monoexponential curve, and  $1/k$  is the time constant ( $T_c$ ). In fact, the PCr resynthesis rate is independent of exercise intensity, as long as muscle pH is close to 7.0 (Yoshida and Watari 1993; Hamaoka et al. 2000c). Therefore, this  $PCr_{T_c}$  is used as the indicator of muscle oxidative capacity (Arnold et al. 1984; McCully et al. 1993; McCully et al. 1994).

The post-exercise PCr resynthesis rate measured by  $^{31}P$ -MRS has been recognized as one of the most reliable parameters for quantifying the rate of oxidative ATP production (Chance et al. 1985; Mahler 1985; Kemp et al. 1993; McCully et al. 1993) because PCr cannot be resynthesized without mitochondrial oxidative phosphorylation (Quistorff et al. 1992). The muscle  $\dot{V}O_2$  at the end of exercise (muscle  $\dot{V}O_{2EX}$ ) was calculated using the equation described below:

$$\text{muscle } \dot{V}O_{2EX} = k \times \Delta PCr$$

While the FBF index was only evaluated as relative to the resting value, resting muscle  $\dot{V}O_2$  (muscle  $\dot{V}O_{2REST}$ ) could be obtained from the PCr breakdown rate during 15 min arterial occlusion (Blei et al. 1993; Hamaoka et al. 1996; Sako et al. 2001). Hence, the ratio of muscle  $\dot{V}O_{2EX}$ /muscle  $\dot{V}O_{2REST}$  can be determined for each subject as a muscle  $\dot{V}O_2$  index. Both muscle  $\dot{V}O_{2EX}$  and muscle  $\dot{V}O_{2REST}$  were expressed as millimoles ATP per second. The absolute values of muscle  $\dot{V}O_2$  (in  $\mu M O_2 s^{-1}$ ) were also calculated using P-to- $O_2$  ratio, which is 6 for in vivo skeletal muscle (Blei et al. 1993; Hamaoka et al. 1996; Sako et al. 2001).

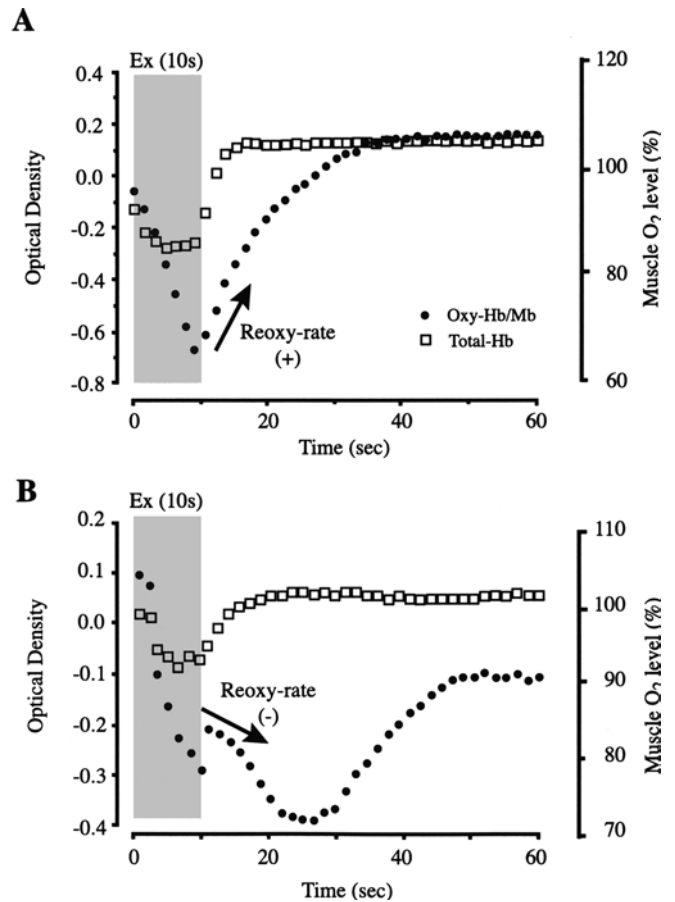
#### Statistics

Using a paired  $t$ -test, we conducted statistical analyses of the differences between the muscle  $\dot{V}O_2$  and FBF indexes. Comparisons of  $PCr_{T_c}$ , muscle  $\dot{V}O_{2EX}$ , the FBF index, and the Reoxy-rate were made using linear correlation. Values are presented as means (SD). Results were considered statistically different at  $P < 0.05$ .

## Results

### Reoxygenation rate after exercise

Figure 2A shows a typical example of changes in Oxy-Hb/Mb and  $Hb_{tot}$  during and after a maximal isometric handgrip exercise. At the beginning of exercise,  $Hb_{tot}$  showed a biphasic response that decreased steeply and remained stable thereafter. On the other hand, Oxy-Hb/Mb dropped linearly until the termination of exercise. After exercise, Oxy-Hb/Mb and  $Hb_{tot}$  rose exponentially. All the subjects but one showed similar kinetics as shown in Fig. 2A. Figure 2B displays the changes in Oxy-Hb/Mb and  $Hb_{tot}$  of the highest oxidative capacity muscle (subject A). Similar to the previous example, Oxy-Hb/Mb dropped rapidly at the beginning of exercise. However, immediately after exercise, although the  $Hb_{tot}$  rose rapidly back to the resting level, Oxy-Hb/Mb continued to deoxygenate until approximately 15 s after exercise and then proceeded to gradually rise. Therefore, this subject's Reoxy-rate indicates a negative value (Table 1). The Reoxy-rate ranged from  $0.53\% s^{-1}$  to  $12.47\% s^{-1}$  [ $7.30(4.7)\% s^{-1}$ ; Table 1]. The Reoxy-rate in relation to the  $PCr_{T_c}$  is shown in Fig. 3. There was a significant positive correlation between the Reoxy-rate and  $PCr_{T_c}$  ( $r^2 = 0.939$ ,  $P < 0.001$ ).



**Fig. 2** Changes in Oxy-Hb/Mb and  $Hb_{tot}$  during and after maximal isometric handgrip exercise in subject G (A) and subject A (B)

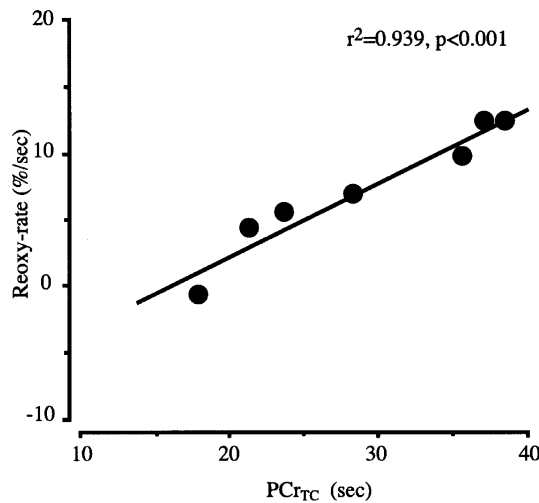
### Measurement of $O_2$ dynamics

The average values of muscle  $\dot{V}O_{2REST}$ , and muscle  $\dot{V}O_{2EX}$  were  $0.0098 \text{ mM ATP s}^{-1}$  and  $0.273 \text{ mM ATP s}^{-1}$ , respectively (Table 1). These values were also equivalent to  $1.65 \mu M O_2 s^{-1}$  and  $45.5 \mu M O_2 s^{-1}$ , respectively, which were calculated using P-to- $O_2$  ratio of 6. At the end of exercise, the muscle  $\dot{V}O_2$  index increased on average by 25-fold compared to the resting value. On the other hand, the FBF index at the end of exercise increased by 3-fold compared to the average resting value. The muscle  $\dot{V}O_2$  index was significantly higher than the FBF index immediately after exercise ( $P < 0.001$ ).

Muscle  $\dot{V}O_{2EX}$  and the FBF index in relation to the Reoxy-rate are shown in Fig. 4. There was a significant correlation between muscle  $\dot{V}O_{2EX}$  and the Reoxy-rate ( $P < 0.05$ ). No correlation, however, was found between the FBF index at the end of exercise and the Reoxy-rate. The  $O_2$  balance, estimated from the separate measurements of the muscle  $\dot{V}O_2$  index and the FBF index (muscle  $\dot{V}O_2$  index/FBF index), for each subject are shown in Table 1. The estimated  $O_2$  balance (muscle  $\dot{V}O_2$  index/FBF index) ranged from 5.62 to 16.08 [ $9.50 (3.63)$ ]. The relationship between the estimated  $O_2$  balance and NIRCws  $O_2$  balance (Reoxy-rate) are shown

**Table 1** Oxygen and nuclear magnetic resonance parameters after maximal isometric exercise in human forearm. *PCr* Phosphocreatine, *Tc* time constant, *Reoxy-rate* reoxygenation rate,  $pH_{min}$  minimum intracellular pH, *muscle  $\dot{V}O_2$*  muscle oxygen consumption, *FBF* forearm blood flow

Subject	$PCr_{Tc}$ (s)	Reoxy- rate (% $s^{-1}$ )	$\Delta PCr$ (mM)	$pH_{min}$	Muscle $\dot{V}O_{2REST}$ (mM ATP $< s^{-1}$ )	Muscle $\dot{V}O_{2EX}$ (mM ATP $< s^{-1}$ )	Muscle $\dot{V}O_2$ index (resting time)	FBF index (resting time)	Muscle $\dot{V}O_2$ index/FBF index
A	17.8	-0.53	6.48	6.87	0.0091	0.352	38.6	2.40	16.08
B	38.3	12.47	11.89	6.88	0.0096	0.221	18.0	3.19	5.64
C	28.2	7.00	7.43	6.82	0.0074	0.226	26.6	3.04	8.75
D	35.5	9.77	7.52	6.88	0.0117	0.276	23.6	4.20	5.62
E	37.1	12.47	7.43	6.85	0.0107	0.201	19.6	2.30	8.52
F	23.5	5.54	7.26	6.82	0.0096	0.279	29.1	2.66	10.94
G	21.1	4.42	8.64	6.85	0.0102	0.350	34.3	3.14	10.92
Mean	28.8	7.3	8.09	6.85	0.0098	0.273	25.4	2.99	9.50
SD	8.3	4.7	1.79	0.03	0.001	0.059	4.49	0.64	3.63

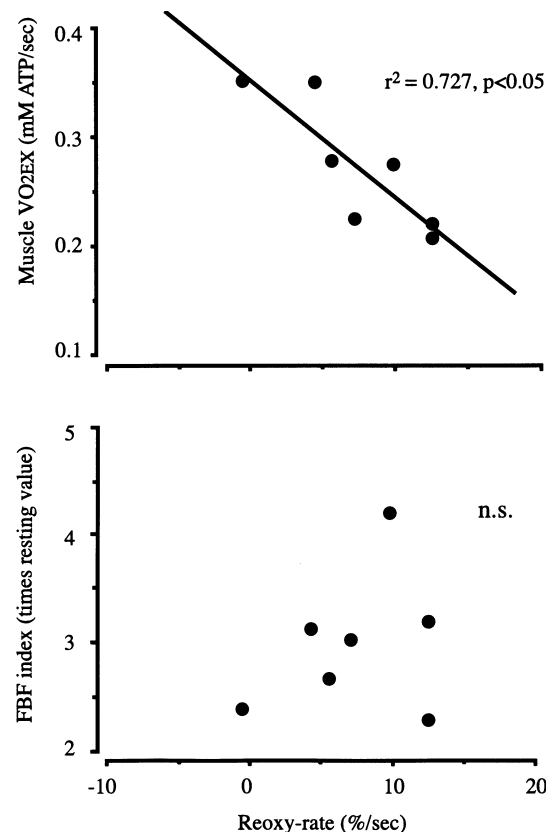


**Fig. 3** Relationship between Reoxy-rate and  $PCr_{Tc}$  after maximal isometric handgrip exercise

in Fig. 5. The estimated muscle  $O_2$  balance closely correlated with the Reoxy-rate ( $r^2=0.820$ ,  $P<0.001$ ). A significant correlation was also found between the muscle  $\dot{V}O_2$  index/FBF index and  $PCr_{Tc}$  ( $r^2=0.799$ ,  $P<0.001$ ).

## Discussion

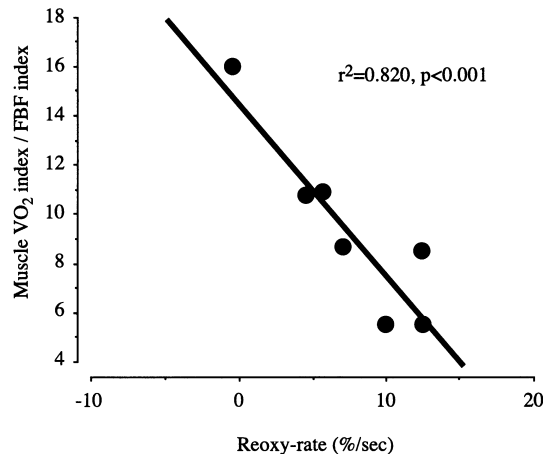
We found a significant relationship between the Reoxy-rate and the  $PCr_{Tc}$  as shown in Fig. 3. Specifically, we found that higher oxidative capacity muscle exhibited slower reoxygenation after exercise. At the end of the exercise, the muscle  $\dot{V}O_2$  exceeded the resting level by approximately 25-fold, while the FBF index exceeded the resting level by only 3-fold on average. These results support our hypothesis that after maximal short-term isometric exercise, when  $O_2$  demand is high and  $O_2$  supply is not fully increased, higher oxidative capacity muscle exhibits slower muscle reoxygenation. Some studies have reported that higher oxidative capacity muscle exhibits a faster reoxygenation rate after exercise (Chance et al. 1992; McCully et al. 1994). This is the first



**Fig. 4** Muscle  $\dot{V}O_{2EX}$  (A) and FBF index (B) in relation to the Reoxy-rate

report indicating that higher oxidative capacity muscle exhibits a slower reoxygenation rate after exercise.

Also in this study, the estimated  $O_2$  balance from the separate measurement of muscle  $\dot{V}O_2$  and  $O_2$  supply closely correlated with the Reoxy-rate. This suggests that the Reoxy-rate is determined by the balance between muscle  $\dot{V}O_2$  and  $O_2$  supply. Furthermore, the Reoxy-rate was not closely correlated with the FBF index, but instead with muscle  $\dot{V}O_{2EX}$  as shown in Fig. 4. These results imply that the Reoxy-rate after this type of exercise may be influenced more by muscle  $\dot{V}O_2$  than by  $O_2$  supply.



**Fig. 5** Relationship between NIRcws  $O_2$  balance (*Reoxy-rate*) and estimated  $O_2$  balance by separate measurements (*muscle  $\dot{V}O_2$  index/FBF index*) immediately after maximal isometric handgrip exercise

### Reliability of this study

In this study, the average value of muscle  $\dot{V}O_{2\text{REST}}$  was  $0.0098 \text{ mM ATP s}^{-1}$ , which is similar to  $0.008 \text{ mM ATP s}^{-1}$  (Blei et al. 1993),  $0.0073 \text{ mM ATP s}^{-1}$  (Hamaoka et al. 1996), and  $0.0076 \text{ mM ATP s}^{-1}$  (Sako et al. 2001) as previously measured by  $^{31}\text{P}$ -MRS. The muscle  $\dot{V}O_{2\text{REST}}$  ( $0.0098 \text{ mM ATP s}^{-1}$ ) was also equivalent to  $1.6 \mu\text{M O}_2 \text{ s}^{-1}$ . This value is very close to the value of  $1.4 \mu\text{M O}_2 \text{ s}^{-1}$  from Harris et al. (1975). The average value of muscle  $\dot{V}O_{2\text{EX}}$  in this study was  $0.273 \text{ mM ATP s}^{-1}$ . This result is similar to  $0.28 \text{ mM ATP s}^{-1}$  obtained from average muscle  $\dot{V}O_2$  following fully excited muscle contraction (Blei et al. 1993). These results demonstrate that our study's muscle  $\dot{V}O_2$  results support the validity of quantitative values. These results also indicate that the finger flexor muscles, that served as agonist muscles in this study, were maximally activated.

According to several studies, a severe decrease in intracellular pH and a restriction of mitochondrial oxidative phosphorylation causes a delay in the PCr resynthesis rate (Arnold et al. 1984; Iotti et al. 1993; Takahashi et al. 1995). Following a non-steady-state exercise, a delayed  $\text{pH}_{\text{min}}$  response may be observed (Vestergaard-Poulsen et al. 1995). This phenomenon is also supported by the creatine kinase reaction with a rapid PCr resynthesis. In this exercise protocol, however, all of the  $\text{pH}_{\text{min}}$  values were above 6.82 (Table 1) and therefore did not restrict mitochondrial oxidative phosphorylation (Takahashi et al. 1995). Some studies have demonstrated a linear relationship between the PCr resynthesis rate and mitochondrial enzyme activities in human skeletal muscle (McCully et al. 1993; Mizuno et al. 1999). Therefore, the time constant for PCr recovery in the experiment should be considered as an indicator of skeletal muscle oxidative capacity.

$O_2$  balance estimated from separate measurements of muscle  $O_2$  consumption and  $O_2$  supply

A significant linear relationship between the estimated  $O_2$  balance and NIRcws  $O_2$  balance (*Reoxy-rate*) was found as shown in Fig. 5. This correlation demonstrates that the estimated  $O_2$  balance from the separate measures of  $O_2$  supply and demand corresponds with the NIR method of  $O_2$  balance. These separate measurements can provide important insight into the factors determining  $O_2$  balance in muscle, which the *Reoxy-rate* alone cannot achieve.

As mentioned earlier, according to our hypothesis, the  $O_2$  balance after maximal short-term isometric contraction is much more influenced by muscle  $\dot{V}O_2$  than  $O_2$  supply. This study found that the ratio of muscle  $\dot{V}O_2$  index/FBF index of all subjects was above 1.0 as shown in Table 1. Van Beekvelt et al. (2001) also reported a greater increase in muscle  $\dot{V}O_2$  than  $O_2$  supply after isometric handgrip contraction. Moreover, the *Reoxy-rate* closely correlated with muscle  $\dot{V}O_{2\text{EX}}$ , but not with the FBF index (Fig. 4). These results suggest that the *Reoxy-rate* after this type of exercise may be influenced more by muscle  $\dot{V}O_2$  than by  $O_2$  supply. This is because increased  $O_2$  demand promotes greater  $O_2$  extraction from the capillaries.

It is possible that the muscle  $\dot{V}O_2$  index/FBF index is equal to the relative change of the arteriovenous (a-v)  $O_2$  difference. The muscle  $\dot{V}O_2$  index/FBF index at the end of exercise ranged between 5.6 and 16.1 compared to the resting value. These values are higher than the reported (a-v)  $O_2$  difference during maximal exercise (2.7- to 8.8-fold of the resting value) (Hartling et al. 1989, Saltin et al. 1998, Bangsbo et al. 2000). One possible explanation for this is the different type of exercise used in this study. Almost all of these studies that measured (a-v)  $O_2$  difference used dynamic exercise for more than a few minutes to fully activate blood flow. In this study, short-term isometric exercise was used to prevent an increase in blood flow after exercise. As a result, FBF index increased by only 3-fold of the resting value after exercise. Lower BF induces longer red blood cell transit time. This may cause greater  $O_2$  extraction.

Another possible reason for the high muscle  $\dot{V}O_2$  index/FBF index value is that the venous effluent at the in vivo sampling sites was a mixture of blood from exercising and non-exercising tissue. Compared to the blood sampling method,  $^{31}\text{P}$ -MRS and NIRS can specifically measure only dominant active muscle information.

### Factors affecting the reoxygenation rate

$O_2$  inflow to the exercising muscle may be completely interrupted during isometric exercise by increased intramuscular pressure (Saltin et al. 1998; Hamaoka et al. 2000b). Stable  $\text{Hb}_{\text{tot}}$  during isometric exercise also supports blood flow interruption during the exercise

(Fig. 2). These reports suggest that the availability of  $O_2$ , but not of convective  $O_2$  transport, may limit muscle  $\dot{V}O_2$  during isometric exercise.

Several studies have reported that the difference in (a-v)  $O_2$  content does not increase at the onset of exercise (Hughson et al. 1996). However, Bangsbo et al. (2000) found that  $O_2$  extraction increased after a few seconds of exercise by obtaining blood transit time from the capillaries to the collection point of the venous blood sample. Recent studies have demonstrated that the ability to detect human skeletal muscle Mb deoxygenation using  $^1H$ -MRS makes it possible to estimate intracellular  $PO_2$  (Richardson et al. 1995; Tran et al. 1999). Richardson et al. (1995) demonstrated that partial Mb deoxygenation occurs even at 50% of maximum  $O_2$  consumption within 20 s of exercise onset (the time resolution of the MRS system). These findings suggest that the intracellular  $PO_2$  may decrease within a few seconds, which may induce a greater  $O_2$  extraction at the onset of blood reperfusion.

A large number of studies have proposed that HOCmuscle is characterized by high mitochondrial oxidative enzyme activity, high mitochondrial density, and high capillary density (Esbjornsson et al. 1993; McCully et al. 1993; Mizuno et al. 1999). Consequently, at the onset of exercise HOCmuscle may produce a more rapid adjustment in muscle  $\dot{V}O_2$  (Phillips et al. 1995; Hamaoka et al. 1998). In addition, considering the high  $O_2$  utilization at the end of exercise in HOCmuscle, intracellular  $PO_2$  in HOCmuscle may be lower than that of LOCmuscle. Therefore, the  $O_2$  gradient from capillary blood to myocyte at the onset of blood reperfusion may be higher in HOCmuscle than in LOCmuscle. The combination of previous studies with our own present findings suggests that the speed and magnitude of Mb deoxygenation during exercise may be one of the most important factors influencing the Reoxy-rate.

## Summary

This study produced three important findings:

1. Significantly positive correlation between the Reoxy-rate and  $PCr_{Tc}$  suggests that HOCmuscle shows slow reoxygenation after maximal isometric exercise.
2. The Reoxy-rate was not closely correlated with the FBF index, but instead with muscle  $\dot{V}O_{2EX}$ . This result implies that the Reoxy-rate after 10 s of maximal isometric exercise may be influenced greater by muscle  $\dot{V}O_2$  than by  $O_2$  supply.
3. The linear correlation between estimated  $O_2$  balance (muscle  $\dot{V}O_2$  index/FBF index) and NIRCws  $O_2$  balance (Reoxy-rate) suggests that the Reoxy-rate determines the balance between muscle  $\dot{V}O_2$  and  $O_2$  supply.

The present study's results may depend heavily on the exercise protocol. The inverse proportional relationship

between the Reoxy-rate and muscle oxidative capacity should not be applied for other types of exercise protocol (i.e., dynamic or whole body exercise). Compared to other exercises, short-term isometric contraction is easily performed. Also, NIRCws is more portable and inexpensive than the MRS device. Therefore, the Reoxy-rate after short-term isometric exercise, as determined by NIRCws, allows us to easily measure the muscle oxidative capacity.

**Acknowledgements** The authors would like to thank the subjects who participated in the study, Dr Sang Yong Bae for his assistance in completing this project, and Toshio Kimura and Gregg Goldstein for all of their help.

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