Reduced Heterogeneity of Muscle Deoxygenation during Heavy Bicycle Exercise

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ABSTRACT

KIME, R., J. IM, D. MOSER, Y. LIN, S. NIOKA, T. KATSUMURA, and B. CHANCE. Reduced Heterogeneity of Muscle Deoxygenation during Heavy Bicycle Exercise. *Med. Sci. Sports Exerc.*, Vol. 37, No. 3, pp. 412–417, 2005. **Purpose:** This study evaluated heterogeneity of muscle O_2 dynamics in a single muscle during bicycle exercise using an eight-channel near-infrared continuous wave spectroscopy (NIRcws) mapping system. **Methods:** Nine healthy subjects performed bicycle exercise at fixed workloads of 20, 40, 60, 80, and 100% maximal workload for 5 min at each level. Muscle oxygenation in the vastus lateralis (VL) during and after each exercise was monitored using the NIRcws mapping system. Pulmonary O_2 uptake and heart rate were monitored continuously during the experiment. Blood samples were taken to measure blood lactate concentration at 30 s after each exercise stage. **Results:** Half time reoxygenation, the time taken to reach a value of half-maximal recovery, was significantly delayed in distal sites compared with proximal sites of VL. Conversely, muscle deoxygenation for all measurement sites increased incrementally with higher exercise workloads, and no significant difference of deoxygenation level showed within each channel. However, relative dispersion of muscle deoxygenation during exercise significantly decreased when the workload increased. Moreover, relative dispersion of muscle deoxygenation between the subjects also decreased with an increase in the workload. **Conclusion:** Muscle deoxygenation in a single muscle was more heterogeneous at lower exercise workloads, and variations of the muscle deoxygenation heterogeneity between subjects were greater at lower exercise workloads. **Key Words:** MUSCLE O_2 DYNAMICS, NEAR INFRARED SPECTROSCOPY, PULMONARY O_2 UPTAKE, LACTATE

Muscle perfusion is a key parameter of aerobic metabolism under various conditions and has been a popular research subject for many years. It is clear that muscle oxygen consumption (muscle $\dot{V}O_2$) increases linearly in relation to muscle perfusion (14,27). Moreover, it has been also shown that muscle perfusion and muscle $\dot{V}O_2$ are affected by differences in muscle fiber composition, microvascular structure, and motor unit recruitment pattern (21,24). Recently, heterogeneity of muscle perfusion and muscle $\dot{V}O_2$ during exercise in humans has been evaluated using positron emission tomography (PET) (11,15). However, the device is expensive and only produces low time resolution. In addition, the PET device cannot obtain information during dynamic exercise because of movement artifacts. Therefore, it is essentially impossible to monitor heterogeneity of muscle perfusion and muscle $\dot{V}O_2$ during dynamic whole body exercise such as bicycle exercise using PET.

Near infrared continuous wave spectroscopy (NIRcws) was first applied to the study of exercising skeletal muscle in humans in 1992 (1). Since then, NIRcws technology has continually been updated and widely applied to the evaluation of muscle tissue oxygenation during bicycle exercise (2,4,13,25). Muscle oxygenation observed using NIRcws reflects the balance between muscle $\dot{V}O_2$ and O_2 supply in localized muscle as demonstrated by its gradual decrease during incremental exercise, and by its dramatic increase after whole body exercise (1,13). More recently, a NIRcws mapping system has been developed to monitor the functional imaging of muscle tissue oxygenation during exercise (17,22,26). However, most of the NIRcws mapping studies were conducted during static exercise due to reduced signal to noise ratio during dynamic exercise. We developed a NIRcws mapping system that enables monitoring of muscle deoxygenation during bicycle exercise, and detailed information of the mapping system has already been published (17). The purpose of this study was to evaluate heterogeneity of muscle deoxygenation and reoxygenation in a single muscle during and after bicycle exercise at specific intensities using an eight-channel NIRcws system.

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METHODS

Approach to the problem and experimental design. To study heterogeneity in muscle deoxygenation and reoxygenation, a multichannel NIRcws probe was placed longitudinally on the VL muscle to measure muscle deoxygenation and reoxygenation during and after bicycle exercise of varying intensities. The independent variables included time and exercise workloads, whereas the dependent variables consisted of the percent muscle deoxygenation and the reoxygenation time measured at eight different points on the muscle. The reoxygenation time, which indicates as an index of O_2 supply to the muscle, was calculated at each measurement area to compare between distal sites and proximal sites for all workloads. Also, relative dispersion of the muscle deoxygenation in relation to variable workload was evaluated as an index of muscle deoxygenation heterogeneity.

Subjects. Nine healthy volunteers (five males and four females, age: 25 ± 4 yr, height: 170 ± 7 cm, weight: 66.4 ± 8.6 kg) participated in this study. All subjects were involved in various recreational activities, such as soccer, volleyball, jogging, and cycling, 2–3× wk^{-1}, but none were trained athletes. All subjects were briefed about the experimental protocol, and written informed consent was obtained before the test. The institutional review board of the University of Pennsylvania approved the research protocol.

Experimental protocol. Before the main experiment, all subjects underwent a preliminary incremental bicycle exercise test (Monark 868, Sweden) to obtain their maximal workloads (W_{max}). Male subjects started pedaling at 100 W, and female subjects started at 50 W. The workload was increased by 25 W every 2 min until exhaustion. On another day, subjects performed bicycle exercise tests at 20, 40, 60, 80 and 100% of maximal workload (%W_{max}). The duration of each workload was 5 min, and the order of the exercise tests was randomized with more than 20 min of interval time between each trial. During each test, a pedal frequency of 60 rpm was maintained by keeping time with a metronome. All subjects were able to maintain the required workload for the entire 5 min. The pulmonary O_2 uptake ($\dot{V}O_2$, in STPD), and carbon dioxide production ($\dot{V}CO_2$, in STPD) were assessed breath by breath with an online metabolic system (SensorMedics 2900, Yorba Linda, CA). Peak $\dot{V}O_2$ at each workload was defined as the $\dot{V}O_2$ averaged over the last 20 s of exercise. Heart rate was monitored continuously during the experiment by ECG. Blood samples were taken via fingerstick for blood lactate concentration ([La]) at 30 s after each exercise stage and analyzed by an enzymatic method (Accusport, Mannheim, Germany).

Multichannel NIRcws imager. We used three wavelength light-emitting diode NIRcws. The device was developed to enable high signal-to-noise ratio (S/N) compared with other NIRcws devices (17). The principle of the measurement and the specifications of the NIRcws have been fully described (17). The level of hemoglobin and/or myoglobin (Hb/Mb) oxygenation alters the absorption of the light in muscle tissue. For example, as Hb/Mb is oxygenated, the absorbance at 730 nm decreases and the absorbance at 850 nm increases, providing a different signal. The difference between the signal at 850 nm and 730 nm is defined as oxygenation change (Δ[Oxy]), which should be sensitive to Hb/Mb O_2 saturation and the sum signal to total Hb change (Δ[tHb]).

The probe of the present system consisted of two light sources and six photodiode detectors, resulting in eight channels. The source to detector distance of all channels was 3.0 cm, and the probe covered a 4.5 × 8.5 cm area. The probe was attached to the skin overlying the lower one-third of the vastus lateralis (VL) muscle.

It should be noted that using the NIRcws technique to evaluate muscle energy metabolism during exercise does give rise to some disadvantages. Primarily, NIRcws values do not specifically reflect muscle $\dot{V}O_2$ but rather reflect a dynamic balance between muscle $\dot{V}O_2$ and O_2 supply (12,19). In addition, when monitoring the oxygenation of muscle tissue, measurement sensitivity is impaired by the influence of adipose tissue (8). Therefore, to more accurately evaluate differences in muscle oxygenation between each subject, arterial occlusion was used; this technique involves interrupting arterial blood flow by placing a pneumatic tourniquet on the upper thigh at a pressure of 300 mm Hg. The muscle oxygenation level (m-O_2 level) was expressed relative to the overall change from the resting preoccluded level to the minimum oxygenation level (6,30). Relative dispersion (RD) of muscle deoxygenation heterogeneity across the region was calculated as RD = (SD/mean) × 100% as an index of heterogeneity (11). Also, half time of oxygenation recovery ($T_{1/2\ reoxy}$), the time taken to reach a value of half-maximal recovery, was calculated after each trial (1,5,13).

Statistics. The changes in recorded parameters during the exercise test were analyzed by one-way ANOVA for repeated measurements. Following a significant *F* test, pairwise differences were identified using Tukey's honestly significant difference (HSD) *post hoc* procedure. When appropriate, significant differences were also identified using Student's paired *t*-tests. The significant level was set at $P < 0.05$.

RESULTS

Whole body responses during each exercise. Mean data describing cardiopulmonary responses are presented in Table 1. $\dot{V}O_2$ gradually increased at higher workloads, and the $\dot{V}O_2$ was well fitted by straight lines with mean slope 13.2 ± 0.5 mL $O_2 \cdot W^{-1} \cdot min^{-1}$ ($r^2 = 0.999$; $P < 0.001$; correlation not shown). HR also increased linearly at higher workloads. [La] started to accumulate at 60%W_{max} and increased significantly with higher workloads.

Muscle reoxygenation after each exercise. Because bicycle exercise elicits a great increase in cardiac output and blood flow to the working muscle, it is speculated that the $T_{1/2}$ after the exercise may reflect the "washing" of O_2 supply (18). Therefore, faster $T_{1/2}$ implies that O_2 supply to the activating muscle may be greater. $T_{1/2\ reoxy}$ of every channel at each exercise trial is illustrated in Figure 2.

TABLE 1. Power output, oxygen uptake, heart rate, and blood lactate concentration at all work rates.

	Pre	20% W_{max}	40% W_{max}	60% W_{max}	80% W_{max}	100% W_{max}
Work rate (W)	—	37.2 ± 3.6	74.4 ± 7.1	111.7 ± 10.7	148.9 ± 14.2	186.1 ± 17.8
$\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	4.7 ± 0.2	14.1 ± 0.7	21.5 ± 0.7	28.5 ± 1.1	37.0 ± 1.2	44.5 ± 1.7
HR (bpm)	65 ± 2	98 ± 3	114 ± 2	134 ± 3	155 ± 3	175 ± 2
La ($mM \cdot L^{-1}$)	1.5 ± 0.1	1.6 ± 0.2	2.1 ± 0.2	2.9 ± 0.2	4.9 ± 0.6	8.4 ± 0.9

The $T_{1/2\ reoxy}$ at preexercise (Pre) was indicated as $T_{1/2\ reoxy}$ after 3 min of arterial occlusion. Also, $T_{1/2\ reoxy}$ at 20%W_{max} could not be measured because muscle deoxygenation did not progress enough to obtain $T_{1/2\ reoxy}$ due to hyperperfusion during the exercise. Therefore, $T_{1/2\ reoxy}$ at 20%W_{max} was neglected in this study.

The $T_{1/2\ reoxy}$ was progressively delayed from distal sites (ch 1, 2) to proximal sites (ch 7, 8) of VL as shown in Figure 2. On the other hand, there were no differences of $T_{1/2\ reoxy}$ between medial and lateral sides at the same transverse level for all workloads (Fig. 2).

In addition, $T_{1/2\ reoxy}$ for all measurement sites was prolonged at higher workloads (Fig. 2). Moreover, $T_{1/2\ reoxy}$ on eight measurement sites at each workload were averaged to obtain a relationship between $T_{1/2\ reoxy}$ and [La]. The correlation between mean $T_{1/2\ reoxy}$ and [La] was represented by a single exponential curve (Fig. 3).

Muscle deoxygenation at various work intensities and its heterogeneity. Muscle deoxygenation changes at various workloads are illustrated in Figure 4. Muscle deoxygenation for all measurement sites increased incrementally with higher exercise workloads, and the deoxygenation level showed no significant difference among each channel.

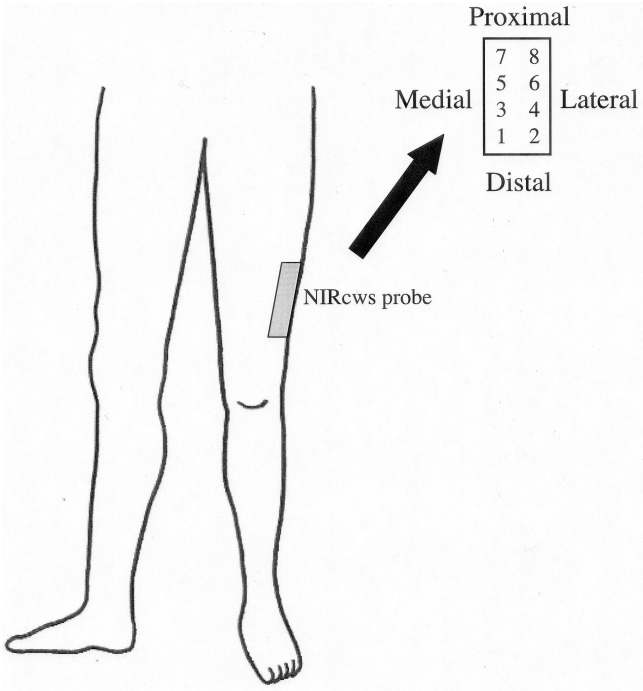

FIGURE 1—Schematic representation of NIRcws probe position. The probe was attached on the vastus lateralis (VL) muscle. The channels were arranged so that channels 1 and 2 were at the distal site and odd-numbered channels were on the medial site of left VL.

RD of muscle deoxygenation in VL during exercise is depicted in Figure 5. RD of muscle deoxygenation significantly decreased at higher workloads. Moreover, error bars (standard deviation) of the RD also dramatically decreased with each increased workload indicating more uniform RD between each subject (Fig. 5).

DISCUSSION

We investigated muscle O_2 dynamic heterogeneity in a single muscle during and after bicycle exercise at various workloads using an eight-channel NIRcws system. The study reveals that muscle deoxygenation in a single muscle was more heterogeneous at lower exercise workloads, and individual variations of the muscle deoxygenation heterogeneity were greater at lower exercise workloads. Recently, some PET studies demonstrated the heterogeneities of muscle O_2 consumption and muscle perfusion during isometric exercise (11) and after exercise (20), but not during dynamic exercise because of the movement artifacts. Laaksonen and colleagues (15) reported on the heterogeneity of muscle perfusion during locomotory exercise with PET, but they used limited leg extension that isolated dynamic muscle action and controlled the range of motion to a minimum. As previous studies alluded, it is generally difficult to use bicycle exercise with PET. This is the first study reporting reduced heterogeneity of muscle deoxygenation in a single muscle during bicycle exercise at heavy workloads.

Reoxygenation rate at various power outputs. $T_{1/2\ reoxy}$ after bicycle exercise is often used as an index of deficits of O_2 delivery to the muscle in relation to O_2 demand of the activated muscle (1). In addition, $T_{1/2\ reoxy}$

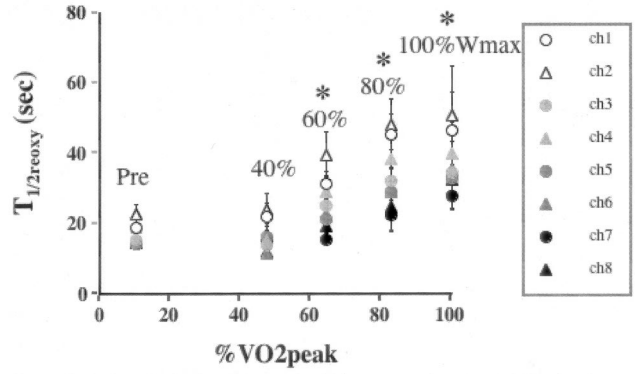

FIGURE 2—$T_{1/2\ reoxy}$ in each measurement site on VL muscle vs percentage of oxygen uptake. *Circles* and *triangles* display medial and lateral sites in VL, respectively. White indicates the most distal sites in the experiment, and the colors are progressively darker to proximal sites (See Fig. 1). * Significantly different between distal (ch 1, 2) and proximal sites (ch 7, 8).

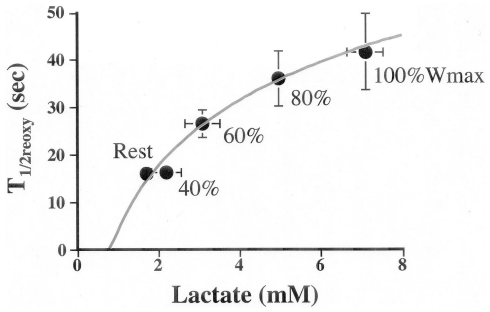

FIGURE 3—Relationship between mean $T_{1/2\ reoxy}$ at each work rate and blood lactate concentration.

after bicycle exercise at workloads that produce low deficits of O_2 delivery such as LT indicates the potential of muscle O_2 delivery (1,5). In this study, $T_{1/2\ reoxy}$ was prolonged at higher workloads, and the correlation between mean $T_{1/2\ reoxy}$ and [La] was represented by a single exponential curve (Fig. 3). These results are similar to the results of Chance et al. (1), which already reported that slower reoxygenations were found at higher workloads. In addition, they suggested that muscle $\dot{V}O_2$ recovery is slowed by higher muscle ADP concentrations and the higher values of ADP would stimulate lactate production. Also, lower pH by H^+ accumulation may inhibit mitochondrial respiration (10). These results contributed to delayed reoxygenation after exercise. Moreover, blood flow during high-intensity exercise was inadequate for the demands of metabolism, which causes prolonged recovery time for muscle $\dot{V}O_2$ after the exercise (23,29). These physiological factors may explain the prolonged reoxygenation after high intensity exercise. Because $T_{1/2\ reoxy}$ at a higher workload is highly influenced by lactic acidosis and delayed blood flow recovery, it is important to take these factors into consideration when using $T_{1/2\ reoxy}$ as an index of postexercise O_2 delivery.

Reoxygenation rates in relation to measurement sites. $T_{1/2\ reoxy}$ at distal sites was more delayed than that at proximal sites (Fig. 2). On the other hand, no specified trend of muscle deoxygenation was observed in any measurement

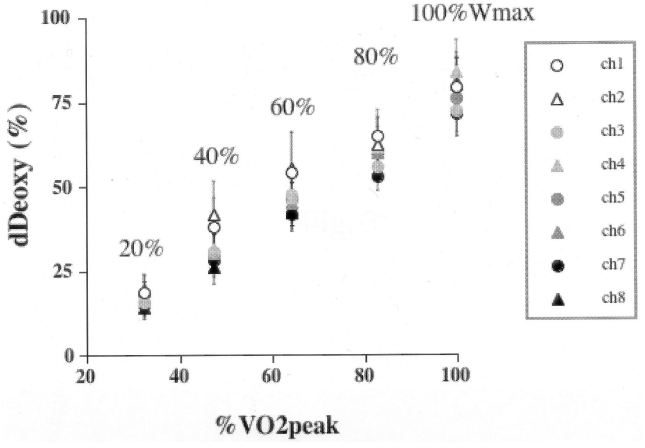

FIGURE 4—Muscle deoxygenation in each measurement site on VL muscle in relation to percentage of oxygen uptake. *Circles* and *triangles* display medial and lateral sites in VL, respectively. White indicates the most distal sites in the experiment, and the colors are progressively darker to proximal sites (see Fig. 1).

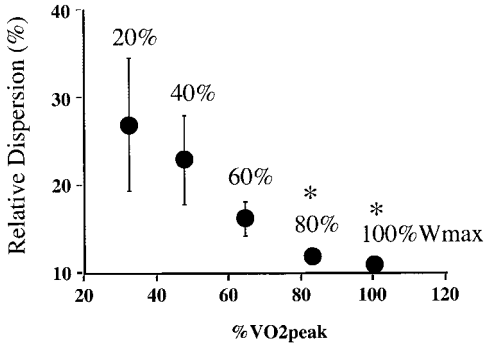

FIGURE 5—Relative dispersion of muscle deoxygenation in VL muscle in relation to percentage of oxygen uptake. * Significantly different from 20%W_{max}.

site at each workload (Fig. 4). These results imply that the differences in the $T_{1/2\ reoxy}$ between distal and proximal sites were not affected by the muscle deoxygenation level in each measurement site.

It has been reported that blood flow and flow velocity of the popliteal artery are lower than those of the femoral artery using Doppler ultrasound in humans (7). Moreover, Mizuno et al. (20) have shown that muscle blood flow in VL in humans was lower in the distal site than in the proximal site using PET. These reports suggest that one of the possible explanations for delayed reoxygenation at distal sites, observed in this study, may be due to lower muscle blood flow. Another possible explanation may be due to differences in muscle fiber type composition. Laxell et al. (16) reported that there were a lower percentage of slow-twitch oxidative fibers in the distal site than in the proximal site in VL in humans. Developed capillary network in slow-twitch oxidative fibers may cause great O_2 delivery to activating muscle and rapid O_2 diffusion to Mb. The delayed reoxygenation at distal sites may be explained by either one or a combination of the above points.

In this study, the measurement probe was attached to the skin overlying the lower one-third of the VL muscle (10–12 cm), and this probe position is usually used for measuring VL muscle by NIRcws (2,4). On the other hand, Puente-Maestu et al. (25) attached a NIRcws probe on the VL muscle 15–20 cm above the patella for measuring reoxygenation kinetics. Because the measurement probe in this study was attached longitudinally over the VL muscle, the distance between distal and proximal sites was 8.5 cm (Fig. 1). Also, the $T_{1/2\ reoxy}$ in distal site (ch 1) was 7.2 and 18.5 s longer than that in proximal site (ch 7) at 40 and 100%W_{max}, respectively (Fig. 2). Hamaoka et al. (5) reported that $T_{1/2\ reoxy}$ after bicycle exercise for triathletes is significantly faster (5.6 s) than for sedentary subjects, and the time difference between athletes and sedentary is smaller than between the distal and proximal sites obtained in this study. Therefore, the probe position is an important point to consider when evaluating muscle oxygenation using NIRcws, especially $T_{1/2\ reoxy}$.

Muscle deoxygenation and its heterogeneity in relation to variable workload. There was a progressive increase in Δ[Deoxy] as the workload increased, suggesting

a greater metabolic demand rather than O_2 supply to exercising muscle (Fig. 4). However, there was no specific trend of Δ[Deoxy] between each measurement site even though reoxygenation speed was slower at the distal site than the proximal site. Niwayama et al. (22) reported that distal sites of VL showed more deoxygenation during isometric knee extension exercise. The reason why a specific trend of O_2 balance in VL during bicycle exercise could not be obtained may be because of different exercise types. Blood flow after static muscular exercise is lower than after dynamic exercise (9,28). In addition, Delp and Laughlin (3) have suggested that increased muscle perfusion is mainly caused by local vascular control systems within the muscle tissue, which primarily affects exercise induced functional hyperemia. The exercise induced functional hyperemia may coordinate blood flow and red cell distribution, and decrease perfusion heterogeneity in skeletal muscle during exercise (11). Moreover, because bicycle exercise consists of contraction and relaxation phases, blood inflow to exercising muscle increases during the relaxation phase between contractions by release of intramuscular pressure. These imply that O_2 supply to the working muscle during bicycle exercise is more uniform than that during isometric exercise.

Although no specific trend of Δ[Deoxy] during exercise was found between each measurement site in relation to workload (Fig. 4), RD of Δ[Deoxy] significantly decreased as workload increased (Fig. 5). Besides, individual variation of RD of Δ[Deoxy] also dramatically decreased as workload increased (Fig. 5). These findings imply that as workload increased, muscle deoxygenation during exercise is less heterogeneous not only in a single muscle but also between individual variations. Because the muscle deoxygenation represents a dynamic balance between muscle $\dot{V}O_2$ and O_2 supply, less heterogeneity of muscle deoxygenation at higher workloads may be explained by one of or a combination of the following points: (a) enhanced muscle perfusion by capillary recruitment and/or micro vascular dilation, and (b) more uniform recruitment of different muscle parts.

SUMMARY

This study demonstrated that $T_{1/2\ reoxy}$ was more delayed at distal sites than proximal sites in the lower one-third of the VL muscle even though there was no specified trend of muscle deoxygenation in each measurement site. In addition, muscle deoxygenation in a single muscle was more heterogeneous at lower exercise workloads, and individual variations of the muscle deoxygenation heterogeneity were greater at lower exercise workloads. Although PET can also measure muscle deoxygenation heterogeneity in humans, multichannel NIRcws is more portable, inexpensive, and has higher time resolution. Whereas PET can only monitor after exercise, during isometric exercise, and dynamic knee extension at low exercise intensities, a great advantage of multichannel NIRcws is that this device can also monitor muscle deoxygenation heterogeneity during bicycle exercise. In addition, because the sampling rate of the multichannel NIRcws system is fast, the system may be highly beneficial for mapping of muscle O_2 deoxygenation heterogeneity during dynamic whole body exercise.

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