

# Unchanged Muscle Deoxygenation Heterogeneity During Bicycle Exercise After 6 Weeks of Endurance Training

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**Abstract** The purpose of this study was to examine the changes in muscle oxygen saturation ( $\text{SmO}_2$ ) level and its heterogeneity after 6 weeks of endurance training using multi-channel near infrared spatially resolved spectroscopy ( $\text{NIR}_{\text{SRS}}$ ). Nine healthy subjects participated in this study (Male = 6, Female = 3, age:  $27 \pm 5$  years, height:  $168.7 \pm 7.4$  cm, weight:  $62.4 \pm 12.4$  kg). The subjects performed a 30 W ramp incremental bicycle exercise test until exhaustion before and after endurance training. The  $\text{NIR}_{\text{SRS}}$  probe was attached to the left vastus lateralis muscle along the direction of the long axis. The subjects performed bicycle exercise for 30 min/day, 3 days/week for 6 weeks. The work rate during training was set at  $60\% \dot{V}\text{O}_{2\text{peak}}$  and increased every  $5\% \dot{V}\text{O}_{2\text{peak}}$  when the subjects could maintain the work rate three times consecutively. After training,  $\dot{V}\text{O}_{2\text{peak}}$  was significantly increased (Pre:  $42.7 \pm 9.9$  ml/kg/min, Post:  $52.3 \pm 7.2$  ml/kg/min,  $p < 0.001$ ) and the mean  $\text{SmO}_2$  within measurement sites at  $\dot{V}\text{O}_{2\text{peak}}$  was significantly decreased (Pre:  $56.1 \pm 1.1$  %, Post:  $53.3 \pm 2.2$  %,  $p < 0.05$ ). Conversely, the heterogeneity of the  $\text{SmO}_2$  during exercise was not changed by training. These results suggest that the functional heterogeneity of  $\text{O}_2$  balance did not change due to endurance training, and the  $\text{O}_2$  balance heterogeneity may not interfere with  $\text{O}_2$  exchange in the activating muscle in healthy individuals.

## 1 Introduction

We have previously reported that muscle deoxygenation level at exhaustion was negatively correlated with peak oxygen uptake (peak  $\text{VO}_2$ ), and the results suggest that  $\text{O}_2$  availability may be enhanced in higher oxidative capacity muscles. Basically, blood flow increases in relation to local metabolic rate [1], and the  $\text{O}_2$  balance is distributed heterogeneously in a single muscle [2, 3, 4]. The

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nonuniform distribution of  $O_2$  balance may interfere with  $O_2$  exchange in the activating muscle [5]. We hypothesized that muscle oxygen extraction may be enhanced by endurance training, and the adaptation will lead to muscle oxygen saturation ( $SmO_2$ ) distribution in the activating muscle being more homogeneous at higher work rates as nonuniform  $O_2$  balance distribution may interfere with  $O_2$  exchange in the activating muscle. There have been no studies reporting the changes in muscle deoxygenation heterogeneity by endurance training in an exercising muscle. Therefore, we examined the changes in muscle oxygen saturation ( $SmO_2$ ) level and its heterogeneity after 6 weeks of endurance training using multi-channel near infrared spatial resolved spectroscopy ( $NIR_{SRS}$ ).

## 2 Methods

### 2.1 Subjects

Nine healthy untrained volunteers (six males and three females, age:  $27 \pm 5$  year height:  $168.7 \pm 7.4$  cm, weight:  $62.4 \pm 12.4$  kg) participated in this study. All subjects were briefed about the experimental protocol, and written informed consent was obtained before the experiment. The institutional review board of the Tokyo Medical University approved the research protocol.

### 2.2 Experimental Design

The peak oxygen uptake ( $\dot{V}O_{2peak}$ ) and  $SmO_2$  distribution in the vastus lateralis (VL) muscle were determined before and after 6 weeks of endurance training. As for the endurance training, the subjects performed bicycle exercise for 30 min/day, 3 days/week for 6 weeks. The work rate during training was set at 60%  $\dot{V}O_{2peak}$  and increased every 5%  $\dot{V}O_{2peak}$  when the subjects could maintain the work rate three times consecutively.

The subjects performed an incremental bicycle exercise until exhaustion to determine the  $\dot{V}O_{2peak}$  and muscle deoxygenation heterogeneity in the VL muscle. During the test, pulmonary  $\dot{V}O_2$  and carbon dioxide production ( $VCO_2$ ) were assessed breath-by-breath with an online metabolic system (AE-300 Minato, Japan). Pedal frequency of 60 rpm was maintained by keeping time with a metronome.

### 2.3 Multichannel $NIR_{SRS}$ System

We used two wavelength light-emitting diode  $NIR_{SRS}$  (ASTEM Co, Japan). The probe of the present system consisted of two light sources and one photodiode detector, and the optode distance was 20 and 30 mm, respectively. The measurement probes were attached to the left VL muscle along the direction of the long

axis. The eight measurement probes were arranged vertically and the most distal site was channel 1 and the most proximal site was channel 8. As leg length is shorter in females than in males, the seven measurement probes were used to cover the left VL muscle in the female subjects (Fig. 1). The  $SmO_2$  was derived from the relative absorption coefficients obtained from the slope of light attenuation over a distance measured at two focal points from the light emission. The relative absorption coefficients are converted to relative concentrations of oxygenated and deoxygenated-Hb. Therefore, this device can essentially calculate tissue oxygen saturation. A previous study has reported that fat layer thickness affects  $SmO_2$  [6]. In contrast, Niwayama et al. [7] recently reported that the  $SmO_2$  can be quantified by the correction of fat layer thickness effects and the specifications of the  $NIR_{SRS}$  were fully described. In this study, we measured fat layer thickness on each measurement site in VL muscle to correct these effects using an ultrasound device (LogiQ3, GE-Yokokawa Medical Systems, Japan) by placing an ultrasound probe on the same sites as the  $NIR_{SRS}$  probes had been placed. The  $SmO_2$  at rest and at  $VO_{2peak}$  were defined as the  $SmO_2$  averaged over the last 5 s of each period.

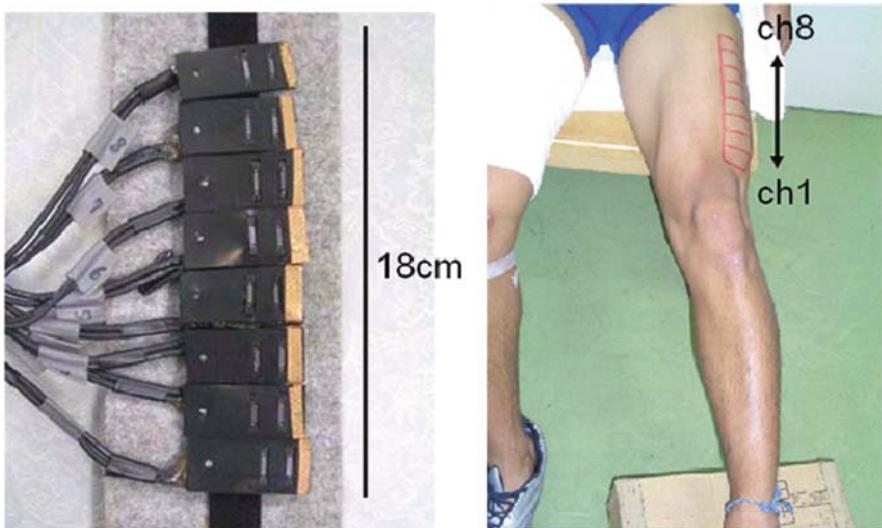


Fig. 1  $NIR_{SRS}$  probes (*left*) and the probe position (*right*)

Relative Dispersion (RD) of  $StO_2$  was calculated as  $RD = (SD/Mean) * 100$  (%) as an index of heterogeneity.

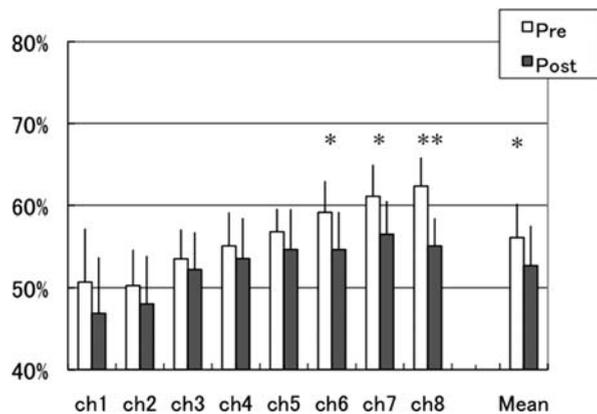
## 2.4 Statistics

All data are expressed as means  $\pm$  SD. All parameters during exercise were compared among pre- and post-endurance training by using a paired *t*-test.

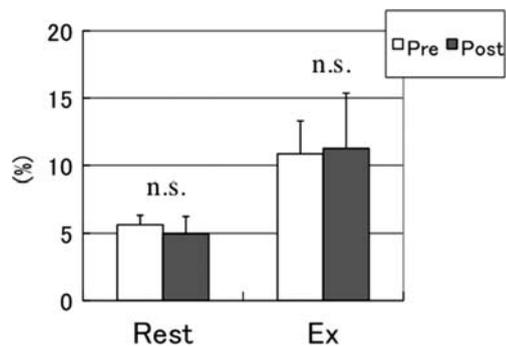
Also, the RD at rest and  $VO_{2peak}$  were compared using a paired  $t$ -test. The level of significance was set at  $p \pm 0.05$ .

### 3 Results

The  $VO_{2peak}$  was significantly enhanced by 6 weeks of endurance training (Pre:  $42.7 \pm 9.9$  ml/kg/min, Post:  $52.3 \pm 7.2$  ml/kg/min, 22.5 % up-regulation,  $p < 0.001$ ). At rest, the  $SmO_2$  of each measurement site in the VL was not significantly different before or after training (Pre-T:  $70.0 \pm 8.2$  %, Post-T:  $71.5 \pm 4.6$  %, mean  $\pm$  SD). In contrast, the  $SmO_2$  at  $VO_{2peak}$  was significantly lower after training at increasingly proximal sites, but not at the distal sites (Fig. 2). Also, the mean  $SmO_2$  in the VL muscle was significantly lower after training (Pre-T:  $56.1 \pm 9.9$  %, Post-T:  $52.7 \pm 11.9$  %,  $p < 0.05$ ). The RD (an index of heterogeneity) was significantly lower at rest than  $VO_{2peak}$  at both pre- and post-training ( $p < 0.05$ ). The RD was not significantly different between pre- and post-training at each rest period or  $VO_{2peak}$ , respectively (Fig. 3). Fat layer thickness was not significantly different after endurance training (Pre-T:  $4.70 \pm 1.78$  cm, Post-T:  $4.56 \pm 1.65$  cm, mean  $\pm$  SD).



**Fig. 2**  $SmO_2$  in VL muscle at  $VO_{2peak}$  before and after endurance training. \*:  $p < 0.05$ , \*\*:  $p < 0.01$



**Fig. 3** Relative dispersion (RD) of  $SmO_2$  in VL muscle before and after endurance training

## 4 Discussion

We investigated changes in  $\text{SmO}_2$  and its distribution in VL muscle at rest and during bicycle exercise at  $\text{VO}_{2\text{peak}}$  by 6 weeks of endurance training using the multi-channel  $\text{NIR}_{\text{SRS}}$  system. This study reveals that muscle deoxygenation in VL was more enhanced at  $\text{VO}_{2\text{peak}}$ , but the muscle deoxygenation heterogeneity in VL was unchanged by endurance training. Recently, some PET studies have demonstrated that muscle  $\text{O}_2$  extraction was higher and muscle blood flow heterogeneity in the exercising muscle was lower in endurance-trained men [2, 3], but not during dynamic exercise because of movement artifacts. In addition, there has been no intervention study to determine whether muscle deoxygenation heterogeneity is reduced by endurance training. This is the first study reporting unchanged muscle deoxygenation heterogeneity in a single muscle during bicycle exercise by endurance training.

Some studies have reported that muscle  $\text{O}_2$  extraction was enhanced after endurance training as demonstrated by a lower venous femoral  $\text{PO}_2$  during exercise [8]. Therefore, it is suggested that  $\text{O}_2$  extraction may be improved in the activating muscle due to endurance training.

In this study, the  $\text{SmO}_2$  in the VL muscle was significantly enhanced after 6 weeks of endurance training, especially at proximal sites, but not at distal sites. One of the reasons may be due to the small sample size. In addition, as the  $\text{SmO}_2$  was already significantly lower at distal sites than proximal sites during exercise at pre-training, the difference in muscle deoxygenation change between distal and proximal sites post-training might be due to the inability to further deoxygenate.

The RD was significantly lower at rest than  $\text{VO}_{2\text{peak}}$  at both pre- and post-training. This implies that the dynamic  $\text{O}_2$  balance between muscle  $\text{VO}_2$  and  $\text{O}_2$  supply is more uniform at rest than during bicycle exercise.

We hypothesized that  $\text{SmO}_2$  distribution in the activating muscle would be more homogeneous at higher work rates after endurance training [9]. However,  $\text{SmO}_2$  distribution in the VL muscle during exercise was not significantly different between pre- and post-training, even though a previous study demonstrated that the muscle blood flow heterogeneity in the exercising muscle was lower in endurance-trained men [2]. The different results in these studies may be explained by the differences of muscle group measurement, measurement depth and measurement devices. As the muscle deoxygenation heterogeneity in the activating muscle was not significantly different after endurance training,  $\text{O}_2$  balance distribution in the activating muscle may not be related to whole body oxidative capacity in healthy individuals.

In conclusion, after 6 weeks of endurance training, the  $\text{SmO}_2$  in VL muscle was significantly enhanced, especially at increasingly proximal sites. In contrast, the  $\text{SmO}_2$  distribution in VL muscle during exercise was not significantly different between pre- and post-training.

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