Comparison of oscillation of oxygenation in skeletal muscle between early and late phases in prolonged exercise

T. YANO, C-S. LIAN, T. ARIMITSU, R. YAMANAKA, R. AFROUNDEH, K. SHIRAKAWA, T. YUNOKI

Laboratory of Exercise Physiology, Faculty of Education, Hokkaido University, Kita-ku, Sapporo, Japan

Corresponding author: T Yano

Tel: +81-011-706-5090

Fax: +81-011-706-5090

E-mail: yano@edu.hokudai.ac.jp

Running head: Oscillation in oxygenation

Summary

The aim of the present study was to compare the oscillations of oxygenation in skeletal muscle between early and late phases in prolonged exercise. During prolonged exercise at 60% of peak oxygen uptake ($\dot{V}o_2$) for 60 min and at rest, oxygenated hemoglobin/myoglobin (Hb/MbO_2) and total Hb/Mb (THb/Mb) were determined by near-infrared spectroscopy in the vastus lateralis. Power spectra density (PSD) for the difference between Hb/MbO_2 and THb/Mb (-HHb/MbO_2: oxygenation) was obtained by fast Fourier transform at rest, in the early phase (1-6 min) and in the late phase (55-60 min) in exercise. Peak PSD in the early phase was significantly higher than that at rest. There were at least three peaks of PSD in exercise. The highest peak was a band around 0.01 Hz, the next peak was a band around 0.04 Hz, and the lowest peak was a band around 0.06 Hz. PSD in the early phase was not significantly different from that in the late phase in exercise. Heart rate (HR) showed a continuous significant increase from 3 min in exercise until the end of exercise. Skin blood flow (SBF) around the early phase was significantly lower than that around the late phase. It was concluded that oscillation of oxygenation in the muscle oxygen system in the early phase is not different from that in the late phase in prolonged exercise despite cardiovascular drift.

Key words

Deoxygenation, Oscillation, Power spectra density, Prolonged exercise, Oxygenation

Introduction

It is well known that glycolysis is a dissipative structure that shows oscillation in yeast (De La Fuente and Cortes 2012). Richard (2003) reported the review of chemical oscillation in yeast. In an anaerobic condition, a chemical substance in glycolysis oscillates by glucose continual input with about a10-min interval. By pulse input of glucose, NAD and NADH oscillate with a 37-sec interval, and their oscillations are gradually attenuated. On the other hand, Satroutdinov *et al*. (1992) described a different type of respiratory oscillation that is apparently not related to the cell. However, the cycle was around one hour. Lloyd (2008) reported that there are three types of respiratory oscillation with periods of about one minute, about 40 min and one day. The timekeeping clock has been shown for oscillations of longer periods but not for oscillation of the shortest period in yeast.

Although yeast is affected by external environment with various conditions, humans have internal environment with constant condition by a homeostasis system. Therefore, in humans, glucose should be maintained at a constant level in the internal environment. In skeletal muscle, energy condition in the internal environment should be adequately controlled even during exercise. For example, carbohydrate is consumed in aerobic and anaerobic conditions, but in the case of a decrease in carbohydrate, free fatty acid is mobilized by hormone secretion (Åstand and Rodahl, 1977) and consequently energy substances can sufficiently be supplied to the skeletal muscle during exercise. We found that there is an oscillation in oxygenation or minus deoxygenation determined by near-infrared spectroscopy (NIRS) in skeletal muscle not only during light exercise (Yano *et al.* 2012) but also at rest and in resting muscle during exercise (Yano *et al.* 2012, Yano *et al*. 2012). A living organ such as skeletal muscle could have oscillation of oxygenation not only during exercise but also in a resting status due to homeostasis of energy substances.

In prolonged exercise, heart rate upward drift occurs, but cardiac output is maintained (Ricardo *et al.* 1999). However, there has been no report about blood flow in active muscle. Skin blood flow is gradually increased with lapse of time (Ricardo *et al.* 1999). Therefore, blood flow into active muscle may be decreased due to the increase in distribution of skin blood flow under the condition of constant cardiac output. In that case, oxygen supply to active muscle could be diminished and consequently may change the oscillation.

The aim of the present study was, therefore, to compare the oscillation in oxygenation in skeletal muscle between early and late phases in prolonged exercise.

Methods

A. Subjects

Six healthy male volunteers participated in the present study. Their age, height and body weight were 20 ± 0.6 yrs, 170 ± 6.3 cm and 62 ± 6.3 kg, respectively. Consent for participation in the study was obtained from all subjects after informing them of the purpose of the experiment, the procedure, and possible risks. The study was approved by the local ethics committee.

B. Experimental Protocol

An electrically braked cycle ergometer (Combi 232C, Japan) controlled by a computer was used in the experiment. The subjects performed ramp exercise to determine peak oxygen uptake (peak $\dot{V}o_2$). The power output was set at 20 watts for 3 min and was increased by 20 watts per minute until the subject was unable to maintain a revolution rate of 60 rpm.

From the linear relationship between oxygen uptake ($\dot{V}o_2$) and work rate obtained in the ramp test, work rate in constant exercise was determined. This work rate corresponded to 60% of peak $\dot{V}o_2$. Each subject rested for 5 min in a recumbent position and changed the position to a sitting position on the bicycle for 5 min. Changing the body position from a recumbent position to a sitting position takes 4 min. After 14 min at rest, constant exercise was performed for 60 min.

C. Measurements

Oxygenated hemoglobin/myoglobin (Hb/MbO_2) and deoxygenated Hb/Mb (HHb/Mb) concentrations in the left vastus lateralis were measured using a near-infrared spectroscopy (NIRS) system (HEO200N, Omron, Japan). The NIRS probe consisted of a light source and an optical detector, with a distance of 3.0 cm between the light source and detector. Dual-wavelength light (760 and 850 nm) emitted from the light source penetrates tissue, where it is either absorbed or scattered, and some of the scattered light returns to the optical detector. The depth of penetration of the radiation is about 1.5 cm (MaCully and Hamaoka, 2000). The sampling frequencies of Hb/MbO_2 and HHb/Mb were 2 Hz. Since Hb/MbO_2 and HHb/Mb are relative values, they cannot be used for comparison of Hb/MbO_2 levels between different persons or between different regions of the body. However, data can be used for elucidating the time trend.

In NIRS, total Hb/Mb (THb/Mb), Hb/MbO_2 and HHb/Mb are obtained by the following equations (Siga *et al*., 1997).

$$\Delta\, Hb/MbO_2 = \Delta OD(840nm) - 0.66\Delta OD\,(760nm)$$

ΔHHb/Mb = -0.59ΔOD(840nm) + 0.80ΔOD(760nm)

ΔTHb/Mb = 0.41ΔOD(840nm) + 0.14ΔOD(760nm).

Yano *et al.* (2012) showed that Hb/MbO_2 was affected by THb/Mb in exercise. They subtracted Hb/MbO_2 from Hb/MbO_2 in order to diminish the effect of THb/Mb. Therefore, we subtracted ΔTHb/Mb from $\Delta Hb/MbO_2$ in the present study. This simply resulted in minus ΔHHb/Mb (oxygenation) as shown in the above equations. Our application is the same as that in recent studies using deoxygenation data (DeLorey *et al.* 2004, Tran *et al.* 1999). It has also been shown from comparative analysis of ^{1}H-NMR and NIRS measurements that HHb/Mb obtained by NIRS expresses signals in skeletal muscle (Tran *et al.* 1999).

Skin blood flow was measured using a laser blood flow meter, Omega Flow FLO-N1 (Omega Wave, Japan). A probe was attached to the right vastus lateralisis muscle. Changes in skin blood flow were measured during rest and exercise. Obtained analog signals were recorded by a data recorder, with analog-to-digital conversion using MacLab/8s (AD Instrument, Castle Hill, USA) for input into a personal computer. Averages for 10 sec in each 3-min interval were used.

Ventilation and gas exchange responses were measured by an on-line computerized breath-by-breath method (AE-280S, Minato Medical Science, Japan). A 2-liter syringe was used to calibrate the system, which was linear throughout a range of 0-600 $l \cdot min^{-1}$ of ventilation. Fractions of O_2 and CO_2 were analyzed using a zirconium solid electrolyte oxygen analyzer and an infrared carbon dioxide analyzer, respectively. The gas analyzers were calibrated by known standard gases (O2: 15.0%, CO2: 5.0%). Then $\dot{V}o_2$ was outputted for 15-sec intervals. Heart rate (HR) was recorded using a heart rate monitor installed in the respiratory gas analyzer.

D. Fast Fourier Transform

Power spectra density (PSD) for the difference between Hb/MbO_2 and THb/Mb was obtained by fast Fourier transform (FFT). PSDs were obtained in the recumbent position for 5 min (rest phase) and in constant exercise from 1 min to 6 min (early phase) and 55 min to 60 min (late phase). In order to visualize the data of low frequency, a low pass filter was used. The pass frequency was set below 0.02 Hz.

E. Statistics

Data are expressed as means $\pm$ standard deviation (SD). The paired t-test was used to examine significant differences between early phase and late phase or rest phase at the same frequency. The paired t-test was also used to examine significant differences between peak PSDs in the rest phase and early phase at the same frequency. Dunnett's test was used to determine the significance in differences between the value at 3 min

and values at other times in exercise for $\dot{V}o_2$, HR, respiratory gas exchange ratio (RER) and skin blood flow. Significant level was set below 0.05.

Results

Peak $\dot{V}o_2$ was 3.44 $\pm$ 0.36 l·min^{-1}. In constant exercise, $\dot{V}o_2$ was 2.07 $\pm$ 0.14 l·min^{-1} and did not change from 3 min to 60 min of exercise (Fig. 1). $\dot{V}o_2$ in constant exercise corresponded to 61 $\pm$ 1.8% of peak $\dot{V}o_2$. HR was 61 $\pm$ 6. 4 beats·min^{-1} in the recumbent position and significantly increased to 67 $\pm$ 6.3 beats·min^{-1} in the sitting position. HR in exercise was 126 $\pm$ 8.6 beats·min^{-1}at 3 min and significantly increased to 151 $\pm$ 13.2 beats·min^{-1} at 30 min and to 160 $\pm$ 11.0 beats·min^{-1} at 60 min of exercise. RER was 0.945 $\pm$ 0.055 at 3 min in exercise. RER at 60 min in exercise (0.895 $\pm$ 0.063) was significantly lower than that at 3 min in exercise. Respiration rate was 17 $\pm$ 2.2 breaths·min^{-1} at rest in the siting position and was 31 $\pm$ 2.7 breaths·min^{-1} at 3 min and then gradually increased to 37 $\pm$ 6.3 breaths·min^{-1} at 60 min in exercise.

Skin blood flow at 3 min in exercise did not significantly change until 12 min but significantly increased after 12 min in exercise (Fig. 2). If values after 12 min in exercise were regarded as standard ones, skin blood flow at 3 and 6 min in exercise was about 80%. Resting value was about 20%.

As shown in Figure 3, Hb/MbO_2 suddenly decreased after the start of exercise and returned to a steady state level. THb/Mb decreased to less than the resting level probably due to the muscle pump after the start of exercise and then increased to a steady state level. Hb/MbO_2 seemed to be affected by THb/Mb. Therefore, we subtracted THb/Mb from Hb/MbO_2. Hb/MbO_2 minus THb/Mb ($-HHb/MbO_2$) was relatively stable in all subjects in the recumbent position but was unstable in some subjects in the sitting position. When exercise was started, $-HHb/MbO_2$ showed a sharp decrease in all subjects. $-HHb/MbO_2$ showed a sharp decrease immediately after the start of exercise and then gradually increased to a steady state in all subjects.

After treatment with a low pass filter, $-HHb/MbO_2$ noise with higher frequency was removed (Fig. 4). From visual inspection, there were no differences in $-HHb/MbO_2$ kinetics in exercise in all subjects. However, it seemed that amplitude in $-HHb/MbO_2$ in the recumbent position was lower than that that in exercise.

As shown in Figures 5 and 6, PSD obtained in the rest phase tended to be lower than that in exercise, but there were significant differences at the same frequency. This is due to the shift of peak PSD. Therefore, we compared peak PSD in the rest phase with that

in the early phase, and found a significant difference in peak PSD. When PSD in the early phase in exercise was compared with that in late phase at the same frequency, there was no significant difference at any frequency. It seemed that there were at least three peaks. The highest peak was a band around 0.01 Hz, the next peak was a band around 0.04 Hz, and the lowest peak was a band around 0.06 Hz.

Discussion

Frequency of oscillation in oxygenation in the muscle

Iotti *et al.* (2010) reported that oscillations of phosphocreatine (CrP) re-synthesis during recovery from light exercise were 0.002-0.025 Hz. Since CrP can recover with oxygen consumption, this frequency range means oscillation of oxygen consumption. There have been only a few studies showing that the oscillation of oxygenation or minus deoxygenation occurs at rest (Yano *et al.* 2012) and during light constant exercise (Yano *et al.* 2012). They reported that peak frequency was a band of 0.0078 Hz. This peak coincides with that in the present study.

The second peak appeared in a band of 0.04 Hz in exercise. Cardiac cycle and respiratory cycle are too fast to explain this relatively low frequency. At rest, 0.04 Hz is known to be derived from sympathetic activity in cutaneous blood perfusion (Soderstrom *et al.* 2003). However, there was not a band of 0.04 Hz at rest. Alternatively, it is known that handgrip exercise activates sympathetic nervous tone with a time delay of exercise onset (Vissing *et al.* 1999) and that muscle sympathetic tone is activated from 60% of peak $\dot{V}o_2$ in dynamic exercise (Saito *et al.* 1999). This may be associated with the second or third peak of PSD.

Interaction of oxygen supply and oxygen consumption in the muscle oxygen system

Hoshi *et al.* (1998) reported detection of oscillation in cerebral hemoglobin oxygenation at intervals of 0.6-5.0 min during the resting period and discussed it as follows: The oscillation was unlikely to be a result of alterations in either systemic circulation or movement affairs since there were differences in the temporal portions of oscillation even between two adjacent brain regions. It was also found in a series of their investigations that oscillation in an oxygenation sate tended to be diminished or even disappeared when subjects were in some special mood states such as tension or vigor. It has therefore been hypothesized that the oscillation is related to spontaneous neural activity. It has been suggested that spontaneous neural activity is responsible for oscillation in a hemoglobin oxygenation state at rest. As a finding in the brain,

oscillation of oxygenation in skeletal muscle occurs in an intrinsic portion of skeletal muscle (Yunoki *et al.* 2012). However, it has been suggested that there is an interaction between oxygen supply and oxygen consumption in an intrinsic portion of skeletal muscle (Yano *et al.* 2012). The present results also suggest an interaction as follows.

Since skeletal muscle is a living organ, various metabolic substances are produced and oxygen pressure is decreased in muscle fibers, although they are at low levels at rest. The metabolic substances might induce upstream signals through endothelial cells and might induce remote vasodilatation (Murrant and Sarelius 2000, Segal *et al.* 2001). In that case, the oscillation due to a certain substance or some substances in muscle might induce oscillation of small arterial vessels. In fact, it is known that there is an endothelial factor in cutaneous blood perfusion signals in a resting state. The frequency has been reported to be 0.01 Hz (Kvernmo *et al.* 1999). This frequency coincides with the present peak frequency of oxygenation ($-HHb/MbO_2$).

A small arterial vessel distributes capillaries to some muscle fibers so that the oscillation of oxygen supply spreads to some activated muscle fibers. Eventually, the oscillations could affect the oscillation in muscle fibers. This means that oscillations among the muscle fibers are synchronized. Thus, oxygen supply and oxygen consumption could interact with each other. Oxygenation is determined by the balance between oxygen supply and oxygen consumption (Chance *et al*. 1992). Therefore, by this interaction, the oscillation in oxygenation determined in the present study could be included in both oxygen supply and consumption.

The amplitude of the highest peak of PSD became higher from the rest status to exercise. Activity level of muscle is considered to be oxygenation level in the present study. However, if synchronization in oxygenations among muscle fibers occurs, since muscle fibers are recruited in exercise, the oscillation could be enlarged by the synchronization by oxygen supply.

Homeostasis mechanism for oxygen supply and energy substance supply to active muscle

In prolonged exercise, cardiac output can be maintained so that HR drift induces a decrease in stroke volume (Ricardo *et al.* 1999). The increase in skin blood flow might decrease muscle blood flow. At 60% of peak $\dot{V}o_2$, muscle sympathetic activity is increased probably due to metabolic substances produced in active muscle (Saito *et al*. 1999). This induces constriction of small arterial vessels in inactive muscle but not small arterial vessels in active muscle due to sympatholysis (Joyner and Thomas

2003). Even at a very low intensity, muscle sympathetic nervous tone is also activated in prolonged exercise (Saito *et al.* 1997). The decrease in central blood volume due to an increase in skin blood volume could induce reflex through cardiopulmonary baroreceptors. By this reflex, activated sympathetic nervous tone would induce further constriction of small vessels in inactive muscle. Furthermore, it has been suggested that heart rate is gradually increased not only by changes in autonomic nervous activities but also by hormonal factors during prolonged exercise (Lian C-S *et al.* 2012). In fact, adrenalin and noradrenalin are increased in prolonged exercise (Horton *et al.* 1998). Since these hormones stimulate α and β receptors, heart rate is increased and small blood vessels are constricted in inactive muscles. Thus, it is thought that oxygen supply for active muscle is maintained at a constant level (muscle oxygen system). As a result, oscillation of oxygenation appeared to remain constant as shown in Figures 4 and 6.

In prolonged exercise, muscle glycogen and blood glucose can be gradually reduced with energy consumption (Åstrand and Rodahl 1977). However, a shift from carbohydrate metabolism to fat metabolism can occur due to the mobilization of free fatty acid and increase in hormone secretion. It is likely that homeostasis of energy supply to active muscle is maintained in prolonged exercise, although there was a change in energy substances as shown in RER. This energy substance supply (energy substance supply system) would also result in maintenance of oscillation.

Conclusions

It is thought that a homeostatic mechanism in the body operates for the muscle oxygen supply system and energy substance supply system in the internal environment during prolonged exercise. It is likely that the muscle oxygen system is more complex than the system of biochemical reactions such as TCA cycle and glycolysis. Oscillation of oxygenation in muscle oxygen system in the early phase was not different from that in the late phase in prolonged exercise despite cardiovascular drift.

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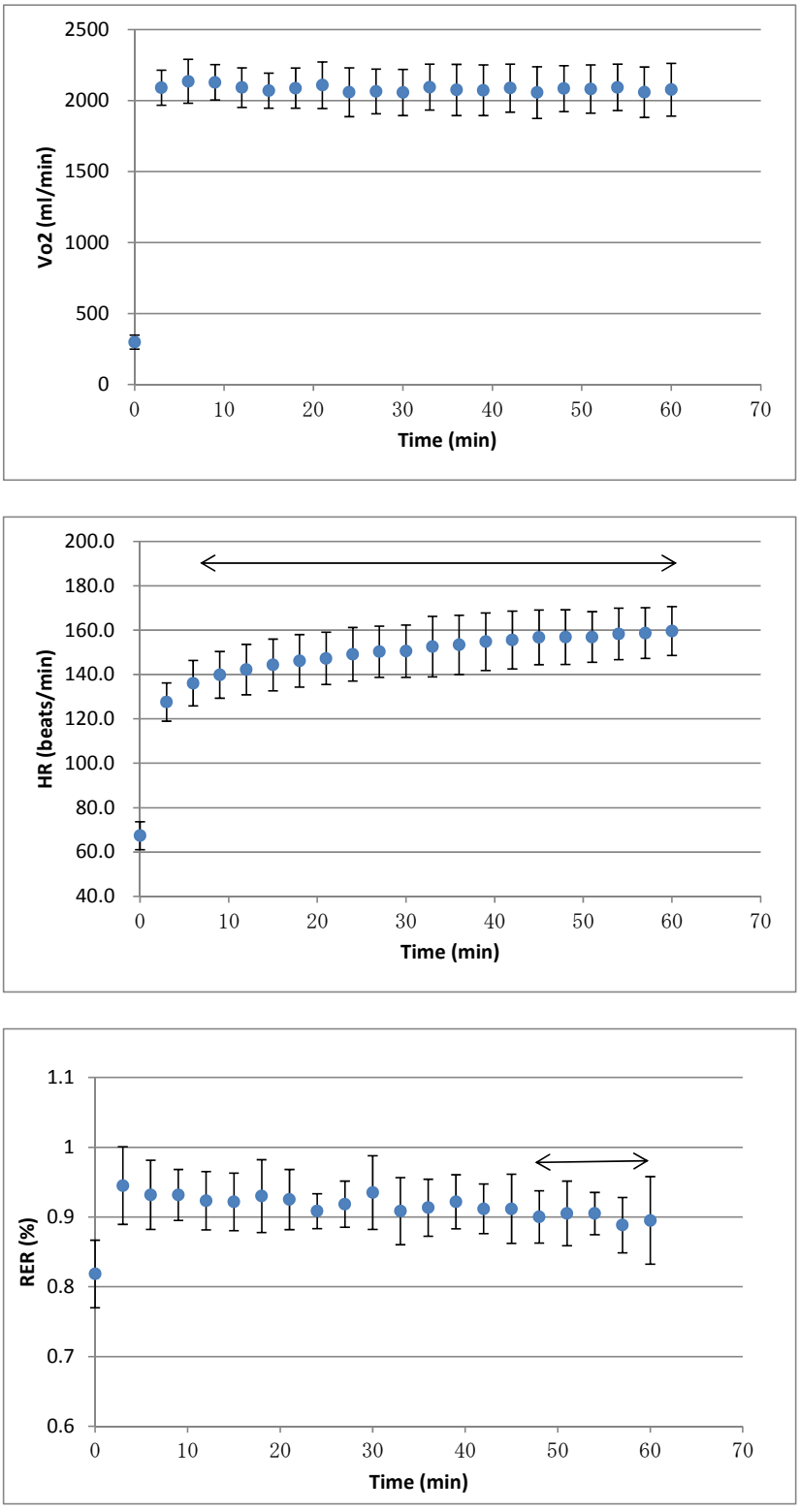


Fig. 1. Oxygen uptake ($\dot{V}o_2$) in the sitting position and that in constant exercise are shown (upper panel). Heart rate (HR) in the sitting position and that in constant exercise are shown (middle panel). Respiratory gas exchange ratio (RER) in the sitting position and that in constant exercise are shown (middle panel). Arrow shows a significant difference from the value at 3 min in exercise.

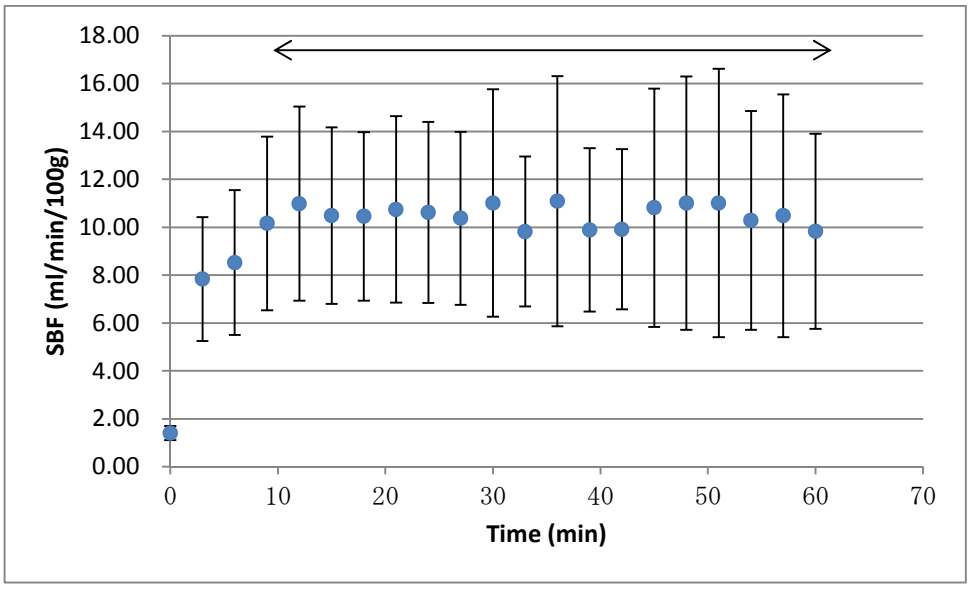


Fig. 2. Skin blood flow in the vastus lateralis in the sitting position and that in constant exercise are shown. Arrow shows a significant difference from the value at 3 min in exercise.

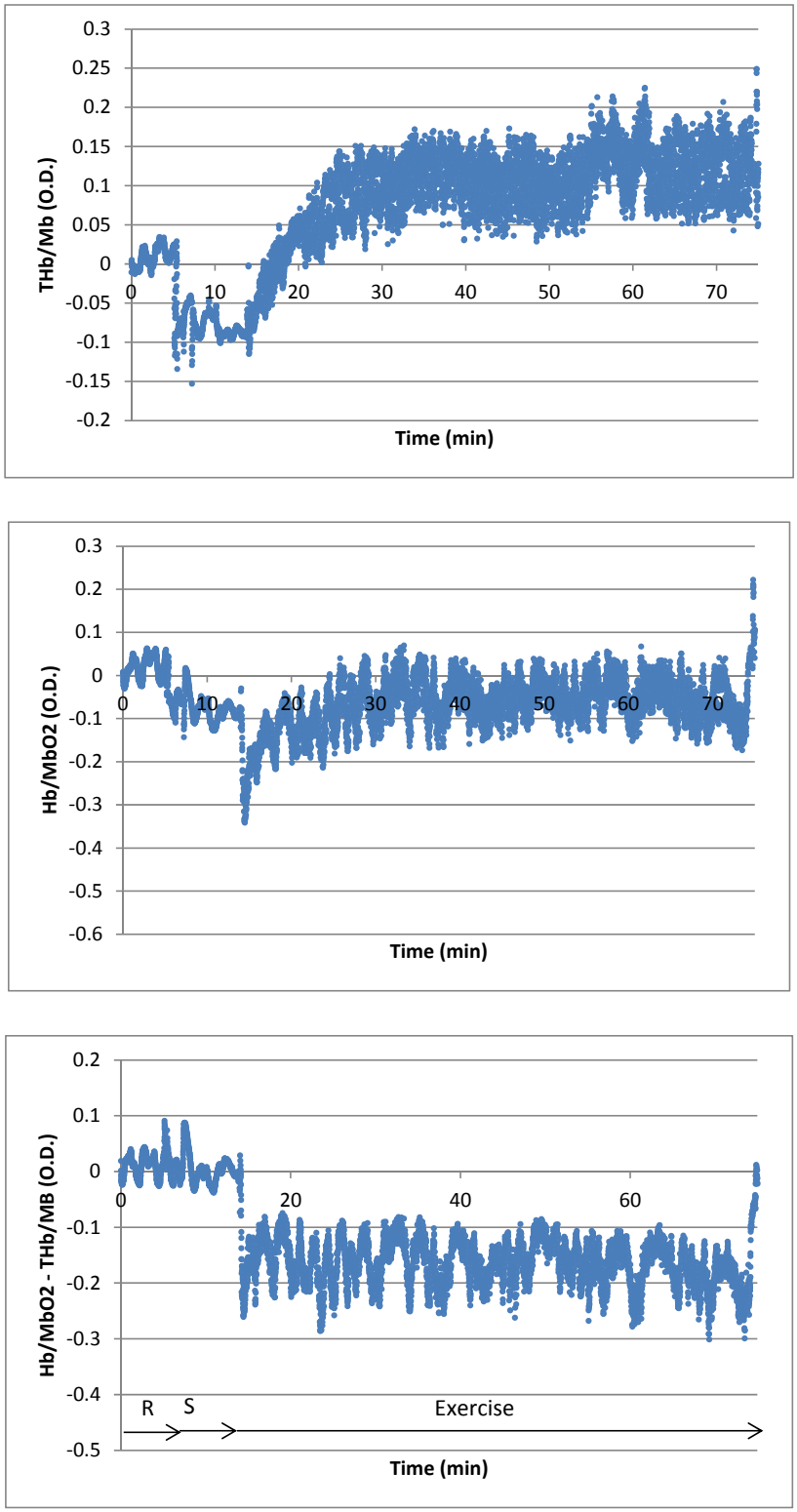


Fig. 3. Typical examples of Hb/MbO_2 (upper panel), THb/Mb (middle panel) and difference between THb/Mb and Hb/MbO_2 (lower panel) are shown. The first period for 5 min was in the recumbent position (R) and the following period for 4 min was used for changing position. The next period for 5 min was in the sitting position (S) on a bicycle ergometer. Constant exercise was started from 14 min and was continued for 60 min.

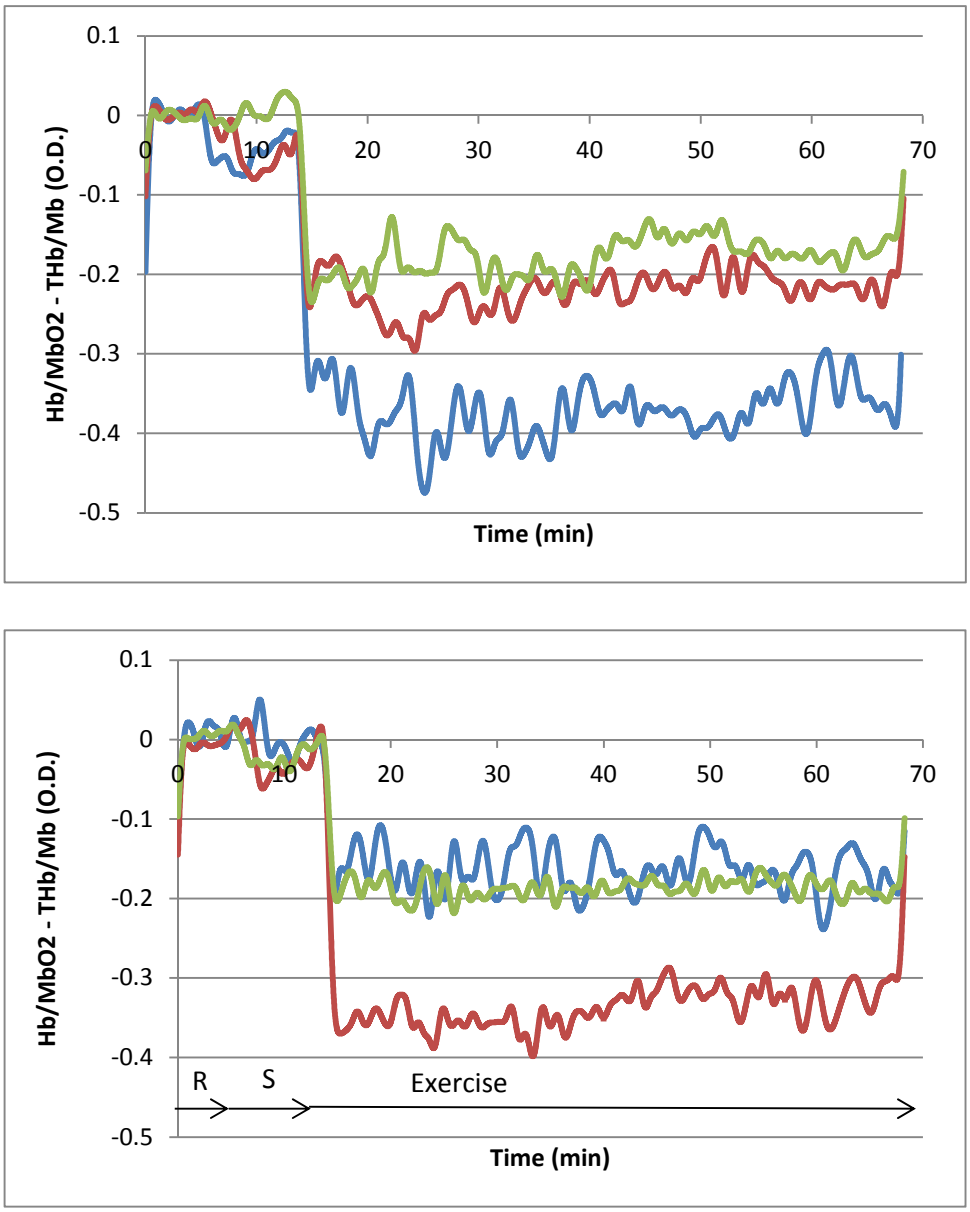


Fig. 4. Data as the differences between THb/Mb and Hb/MbO_2 were treated by a low pass filter. The first period for 5 min was in the recumbent position (R) and the following period for 4 min was used for changing position. The next period for 5 min was in the sitting position (S) on a bicycle ergometer. Constant exercise was started from 14 min and was continued for 60 min. Color lines show changes of individual values.

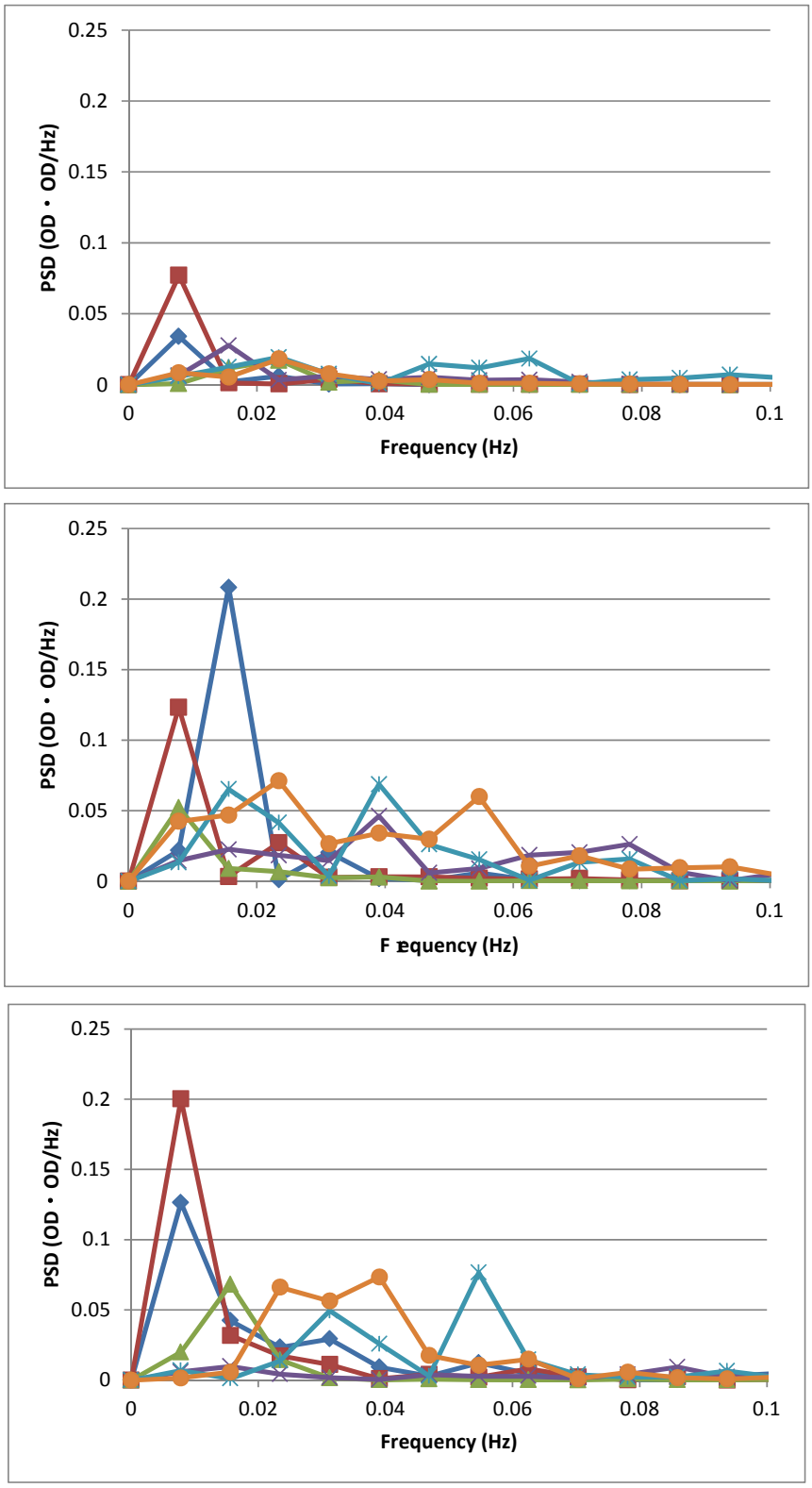
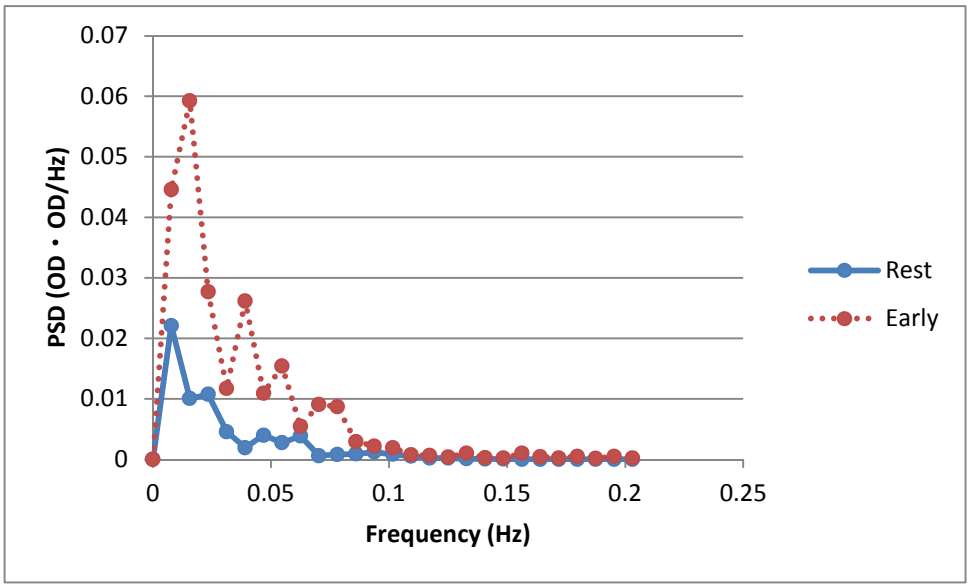


Fig. 5. Individual power spectra densities (PSD) obtained at recumbent rest (upper panel), in the early phase in constant exercise (middle panel) and in the late phase in constant exercise (lower panel) are shown. Color lines show changes of individual values.



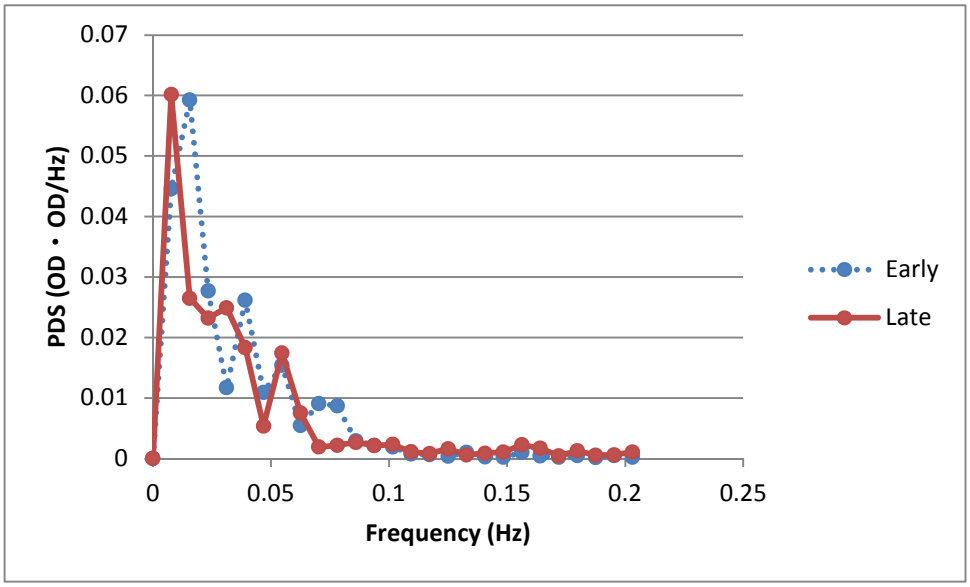


Fig. 6. Averaged PSD is shown in the upper panel (straight line indicating resting values and dotted line indicating exercising values). Averaged PSD is shown in the lower panel (straight line indicating exercising values in the early phase and dotted line indicating exercising values in the late phase).