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# Oscillation of oxygenation in skeletal muscle at rest and in light exercise

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Running head: Oscillation in oxygenation

The aim of the present study was to compare the frequency of oxygenation determined in the vastus lateralis by near-infrared spectroscopy (NIRS) in light exercise with that at rest. A subject rested in a recumbent position for 5 min and changed body position to a sitting position on a cycle ergometer for 9 min. Then exercise with low intensity (work rate of 60% of maximal oxygen uptake) was carried out for 30 min. Total hemoglobin and myoglobin (THb/Mb) suddenly decreased after the start of exercise and gradually increased until 6 min. Oxygenated hemoglobin and myoglobin ($Hb/MbO_2$) suddenly decreased and returned to a steady state after the start of exercise. The difference between $Hb/MbO_2$ and THb/Mb showed a sudden decrease and then a steady state. This difference was analyzed by fast Fourier transform. The peak frequencies of the power spectrum density (PSD) were $0.0169 \pm 0.0076$ Hz at rest and $0.0117 \pm 0.0042$ Hz in exercise. The peak frequency of PSD was significantly decreased in exercise. In exercise, the range of frequencies was expanded. It is concluded that there are oscillations at rest as well as in exercise and that the frequency of peak PSD becomes lower in exercise than at rest.

**Keywords**: oxygenation, oscillation, exercise, near-infrared spectroscopy, power spectrum density

Oscillation has been shown to occur in extreme metabolic conditions such as ischemia or substance depression in different models such as isolated cardiac mitochondria (6, 7), cardiac myocytes (21), neurons (19, 28, 29), and yeast (13-16). It has also been known that dissolved oxygen has oscillation in yeast (22). These results indicated that glycolysis and the TCA cycle are a dissipative structure. Exercise requires at least 10-times greater oxygen consumption than resting muscle. This suggests that oscillation appears in exercise. However, it has been found that oscillation occurs even at rest in humans (31). Although this is a discrepancy, we tried to resolve the discrepancy by switching the concept from a dissipative structure to the muscle organ regarded as a living system (see Discussion section).

Iotti et al. (10) found oscillation of phosphocreatine (PCr) re-synthesis during recovery from exercise in humans. PCr can recover by oxygen consumption. This oscillation reflects the oxygenation characteristics of mitochondria. They also found that the oscillations depend on cytosolic pH. The highest frequency occurs at cytosolic pH>7 and the lowest occurs at pH=6.8. The frequency ranged from 0.002 to 0.025 Hz.

Near-infrared spectroscopy (NIRS) enables determination of hemoglobin and myoglobin oxygenation ($Hb/MbO_2$). It has been found that there are oscillations not only during ramp exercise below the ventilatory threshold but also at rest (31). The frequency was 0.005 Hz. This oscillation may include the effect of oxygen supply to muscle since this oxygenation is determined by oxygen supply and oxygen consumption (4). If oscillation in oxygen supply affects the observed oscillation, two oscillations occurring between two muscle portions should have a certain time delay. This possibility was examined and it was shown that there is time delay but that the time delay shows plus or minus in subjects (32).Therefore, this result means that the oscillation is not only affected by oxygen consumption but also occurs in an intrinsic portion in muscle.

It is hypothesized that the difference between pH at rest and that in exercise due to the difference in lactate levels induces the difference in frequency of oscillation as shown the results obtained by Iotti et al. (10). In order to examine this hypothesis, we compared the frequency of oxygenation in light exercise with that at rest.

## Material and Method

### *A. Subjects*

Six healthy male volunteers participated in the present study. Their age, height and body weight were 20 $\pm$ 0.6 yrs, 170 $\pm$ 6.3 cm and 62 $\pm$ 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. 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 and work rate obtained in the ramp test, work rate in constant exercise was determined. This work rate corresponded to 60% peak oxygen uptake. Each subject rested for 5 min in a recumbent position and changed the position to a sitting position on the bicycle for 5 min. For the changing body position from a recumbent position to a sitting position, it takes 4 min. After 14 min at rest position, constant exercise was performed for 30 min.

*C. Measurements*

Oxygenated hemoglobin/myoglobin ($Hb/MbO_2$) concentrations in the left vastus lateralis were measured using a 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 (17). The sampling frequency of $Hb/MbO_2$ was 2 Hz. NIRS is absorbed by hemoglobin and myoglobin. Form changes in the optimal densities ($\Delta OD$), oxy-hemoglobin and myoglobin ($Hb/MbO_2$), deoxy-hemoglobin and myoglobin (HHb/Mb) and total-hemoglobin and myoglobin (THb/Mb) were calculated as following equations.

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

$$\Delta HHb/Mb = -0.59\Delta OD(840nm) + 0.80\Delta OD(760nm)$$

$$\Delta THb/Mb = 0.41\Delta OD(840nm) + 0.14\Delta OD(760nm).$$

Since $Hb/MbO_2$ is a relative value, it 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.

Deep temperature was measured using Core Tem Monitor (Termo). The depth of measurement was 10 mm from the skin. The Core Temp monitor probe was attached to the right vastus lateralis muscle. Changes in deep temperature were measured during

resting and exercise with a sampling rate of 3 min.

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·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 oxygen uptake ($\dot{V}o_2$) was outputted for 15-sec intervals. Heart rate (HR) was recorded using a heart rate monitor installed in the respiratory gas analyzer.

*D. Statistics and Fast Fourier Transform*

Data are expressed as means $\pm$ standard deviation (SD). Paired t-test was used to examine significant difference between resting and exercising values. Significant level was below 0.05. 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 and in constant exercise from 6 min to 16 min.

## Results

Peak $\dot{V}o_2$ was 3.44 $\pm$ 0.36 l/min. In constant exercise, $\dot{V}o_2$ was 2.07 $\pm$ 0.14 l/min and did not change from 3 min to 30 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 in the recumbent position and significantly increased to 67 $\pm$ 6.3 beat/ min in the sitting position. HR in exercise was 126 $\pm$ 8.6 beats/min at 3 min and significantly increased to 150 $\pm$ 11.8 beats/min at 30 min of exercise. Respiration rate was 17 $\pm$ 2.2 breaths/min at rest in the siting position and was 31 $\pm$ 2.7 breaths /min at 3 min and then gradually increased to 37 $\pm$ 5.7 breaths /min frequency/min at 30 min in exercise.

Deep temperature (Tm) in the vastus lateralis rapidly increased up to 9 min and then showed a slow increase (Fig. 2). Tm was 35.3 $\pm$ 0.45 $^{o}$C at rest in the siting position and was 37.4 $\pm$ 0.30 $^{o}$C at 30 min of exercise.

As shown in Figure 3, $Hb/MbO_2$ showed that oscillation was a fairly stable state in all subjects in the recumbent position but unstable state in some subjects in the sitting position. When exercise was started, $Hb/MbO_2$ showed a sharp decrease and returned to a constant level in all subjects. THb/Mb showed a sharp decrease immediately after the start of exercise and then gradually increased to a steady state in all subjects. THb/Mb showed oscillation in the recumbent position and in exercise in all subjects. THb/Mb kinetics was prospectively similar to $Hb/MbO_2$ kinetics in exercise.

When $Hb/MbO_2$ was subtracted by THb/Mb, its kinetics showed a sudden decrease and steady state in exercise. It seemed that the oscillation was reduced in all subjects.

Figure 4 shows power spectra density (PSD) of the difference between $Hb/MbO_2$ and THb/Mb. At recumbent rest, peak frequency of PSD appeared at 0.0169 $\pm$ 0.0076 Hz. In exercise, peak frequency of PSD was 0.0117 $\pm$ 0.0042 Hz. The peak frequency of PSD was significantly decreased in exercise. However, in exercise, range of frequency was expanded. There was a second peak of the band around 0.04 Hz in four subjects (0.31-0.54). The average value of PSD was significantly higher in exercise than at rest on frequency levels of 0.008 and 0.016 Hz.

## Discussion

Hoshi et al. (9) 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. However, there have been only a few studies showing that the oscillation of oxygenation occurs at rest (31) and in an intrinsic portion in skeletal muscle (32).

THb/Mb is an index of total blood volume. THb/Mb suddenly decreased after the start of exercise due to milking action by the muscle pump. Then THb/Mb gradually increased until 6 min after the start of exercise. It is known that an increase in body temperature induces an increase in compliance of peripheral capacity vessels (18). Therefore, venous blood volume is thought to be increased by an increase in muscle temperature. When venous blood volume is increased, total oxygen content in venous blood can be increased. Therefore, we tried to adjust the effect of THb/Mb on $Hb/MbO_2$. Rapid decrease after the start of exercise and gradual decrease to a steady state in are entirely diminished by subtracting THb/Mb from $Hb/MbO_2$.

In NIRS, THb/Mb, $Hb/MbO_2$ and deoxy hemoglobin and myoglobin (HHb/Mb) are obtained by the following equations (24).

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

$\Delta$HHb/Mb = -0.59$\Delta$OD(840nm) + 0.80$\Delta$OD(760nm)
$\Delta$THb/Mb = 0.41$\Delta$OD(840nm) + 0.14$\Delta$OD(760nm).
We subtracted $\Delta$THb/Mb from $\Delta$Hb/$MbO_2$ in the present study. This simply resulted in minus $\Delta$HHb/Mb. It seems that HHb/Mb is a better indication. Our application is the same as that in recent studies using deoxygenation data (5, 27). It has also been known from comparative analysis of $^1$H-NMR and NIRS measurements that HHb/Mb obtained by NIRS expresses signals in skeletal muscle (27).

Since skeletal muscle is a living organ, various metabolic substances are produced or oxygen pressure is decreased in muscle fibers although they are at low level at rest. The metabolic substances could make upstream signal through endothelial cells and could induce remote vasodilatation (20, 25). In this case, the oscillation due to a certain substance or some substances in muscle could conduct an 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 (12). The amplitude of oscillation can be enlarged in exercise probably due to a lack of oxygen in muscle.

A small arterial vessel expands 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 in muscle fibers are synchronized. Thus, oxygen supply and oxygen consumption could interact with each other. Such a system is a closed muscle oxygen system.

The present exercise intensity was 60%. In this exercise intensity, blood lactate becomes from 1 mM at rest to 2-3 mM in the present exercise (1). Below 4.8 mM (74% of peak $\dot{V}o_2$), blood lactate level is maintained at a constant level in cycling exercise (2). This constant blood lactate suggests that lactate level in muscle fibers is constant. In this case, pH in muscle fibers should decrease slightly. The decrease in pH could be maintained in response to a constant level of blood lactate during exercise. The present results of peak PSD clearly showed reduction of frequency in the oxygen muscle system. This is due to cytosolic pH reduction, since Iotti et al. (10) showed that a cytosolic pH decrease induces low oscillation frequency.

There was a second peak in the band around 0.04 Hz in exercise. Although the higher band may be derived from outside of the muscle oxygen system, cardiac frequency (around 2.3 Hz; 140 beats/min) and respiratory frequency (around 0.58 Hz; 35 breaths/min) are too high. In cutaneous blood perfusion, 0.04 Hz is known to be derived from sympathetic activity on the vessel wall at rest (12, 26). Since skin and muscle sympathetic neural outflow activity oscillated coherently over a range of

frequencies (0.01-1 Hz) at rest (3), muscle sympathetic activity might be associated with this frequency. However, at rest, we did not observe this frequency. In exercise, muscle sympathetic nervous tone is activated at 60% of peak $\dot{V}o_2$ (23). This activation can be inhibited in small arteries in active muscle but is preserved in feed arteries (11). Therefore, this oscillation may be associated with that of feed arteries due to sympathetic nervous activation. Thus, since feed arteries are located external to the muscle (30), such a system is thought to be an open system in exercise.

Recent studies on oxygen consumption kinetics at the onset of exercise have focused whether the oxygen consumption is determined by oxygen supply or by an intramuscular mechanism ($O_2$ delivery limitation or $O_2$ availability hypothesis) (8). For examining this hypothesis, one-way direction from cause to result is assumed. Our approach is system analysis. In this system, it is assumed that factors in muscle oxygen system are interacted in each other and that the interactions lead to self-organization of the muscle oxygen system for muscular work. In this system, the interactions are physiological mechanisms.

In summary, there were some oscillations in the resting state as well as in exercise. The peak of frequency in oscillations became lower in exercise than at rest. High frequency band was enlarged in exercise. A muscle oxygen system is a closed system at rest but may need to change to an open system or a system having a strong interaction with a central oxygen transportation system in exercise.

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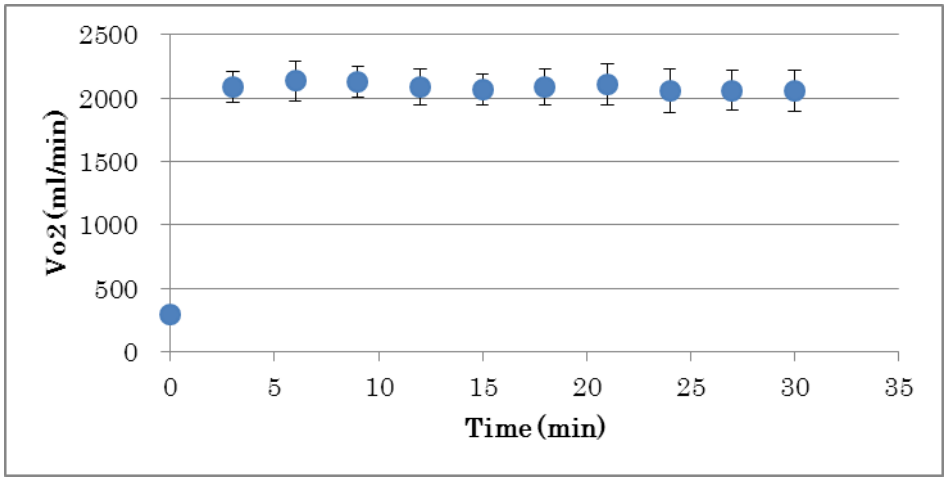
Legends of figures

Fig. 1. Oxygen uptake in the sitting position and that in constant exercise are shown (upper panel). Heart rate in the sitting position and in constant exercise are shown (middle panel). Respiration rates in the sitting position and in constant exercise are shown (lower panel).

Fig. 2. Deep temperatures in the vastus lateralis in the sitting position and in constant exercise are shown.

Fig. 3. Typical examples of $Hb/MbO_2$ (upper panel), THb/Mb (middle panel) and the difference between THb/Mb and $Hb/MbO_2$ (lower panel) are shown. The first period for 5 min was in the recumbent position and the following period for 4 min was used for changing position. The next period for 5 min was in the sitting position on a bicycle ergometer. Constant exercise was started from 14 min and was continued for 30 min.

Fig. 4. Individual power spectra densities (PSD) obtained at recumbent rest (upper panel) and in constant exercise (middle panel) are shown. Averaged PSD is showed in the lower panel (dotted line indicating resting values and straight line indicating exercising values). #: significant difference between PSD at rest and in exercise.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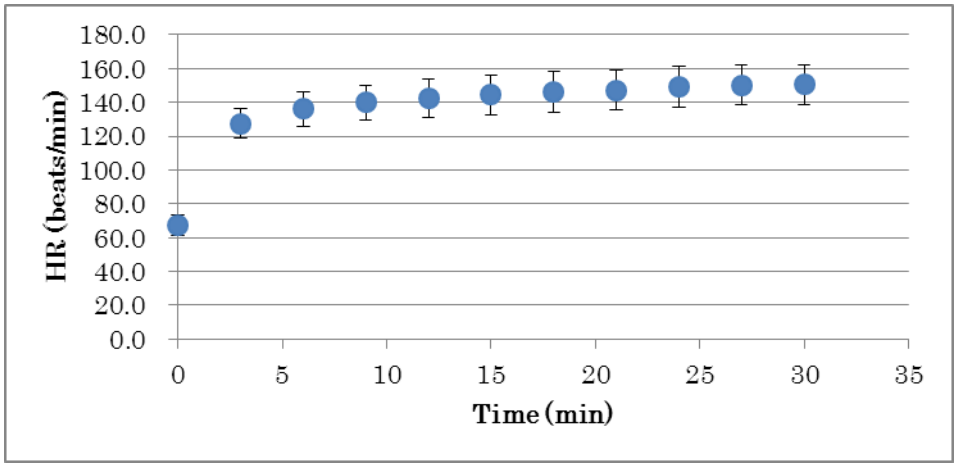
5
10
15
20
25
30
35
Time (min)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80.0
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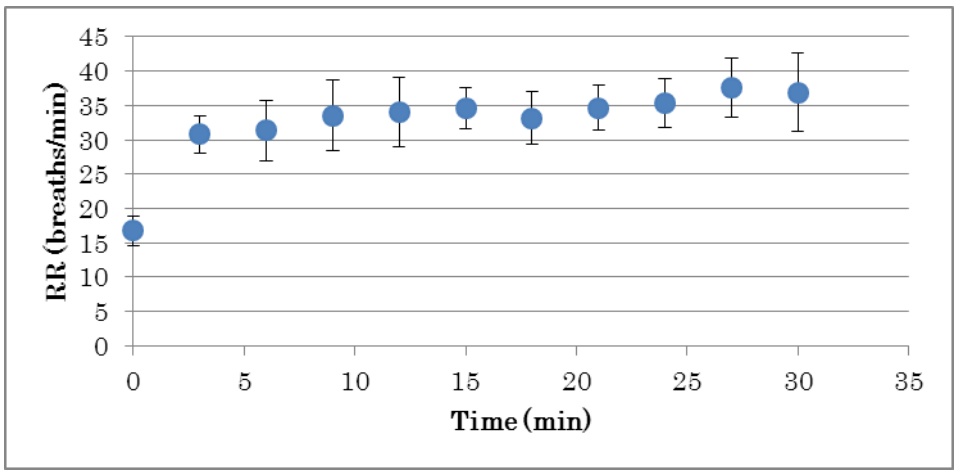
5
10
15
20
25
30
35
Time (min)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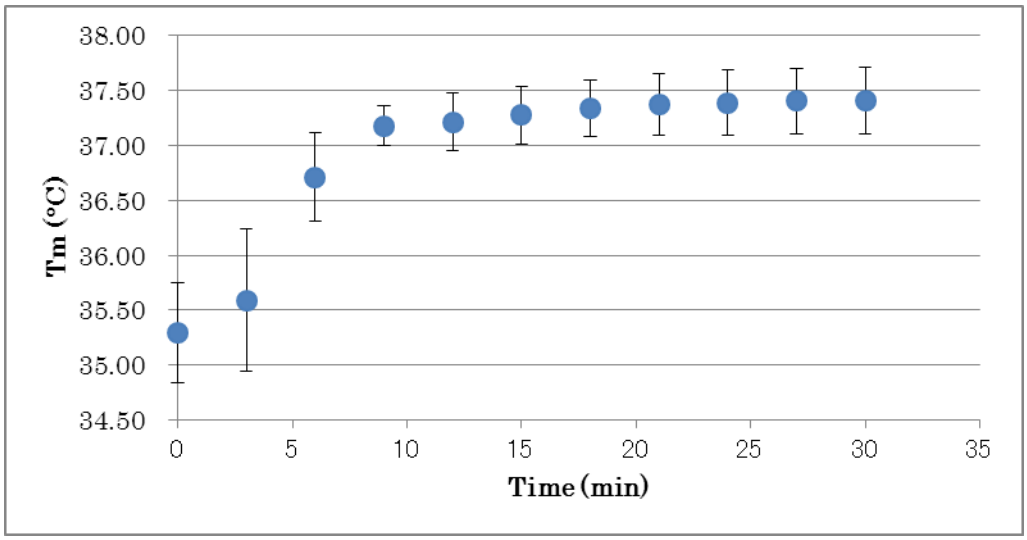
35
Time (min)

Fig. 1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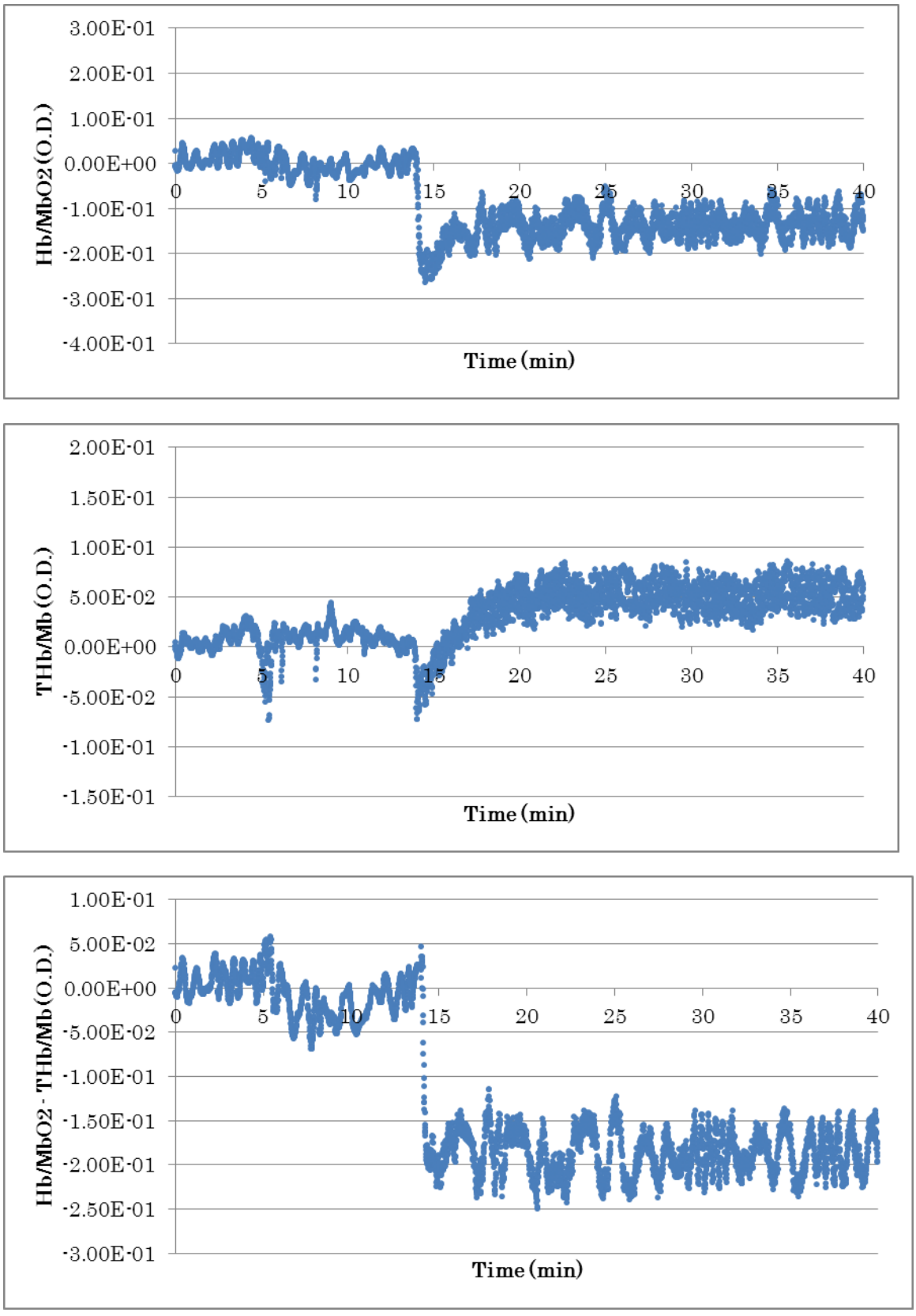
30
35
Time (min)

Fig. 2.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0.00E+00
-1.00E-01
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-3.00E-01
-4.00E-01
Hb/MbO2 (O.D.)
0
5
10
15
20
25
30
35
40
Time (min)
2.00E-01
1.50E-01
1.00E-01
5.00E-02
0.00E+00
-5.00E-02
-1.00E-01
-1.50E-01
THb/Mb (O.D.)
Time (min)
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5.00E-02
0.00E+00
-5.00E-02
-1.00E-01
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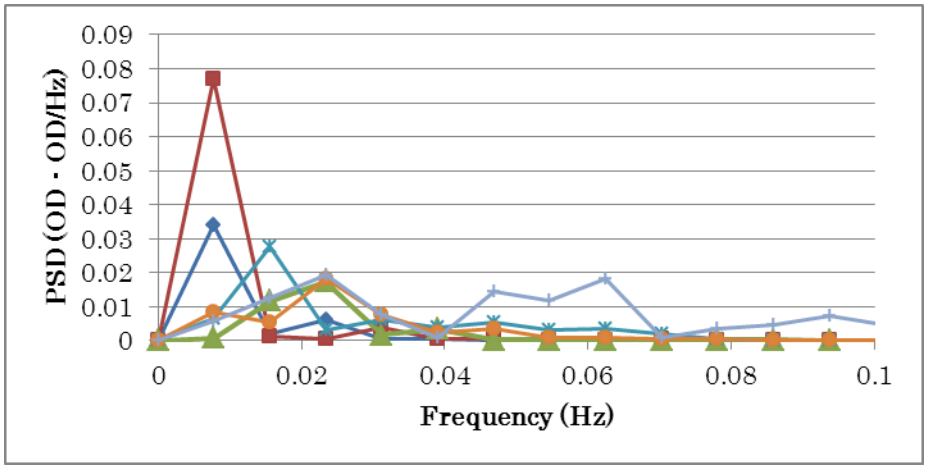
Hb/MbO2 - THb/Mb (O.D.)
Time (min)

Fig. 3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0.02
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0
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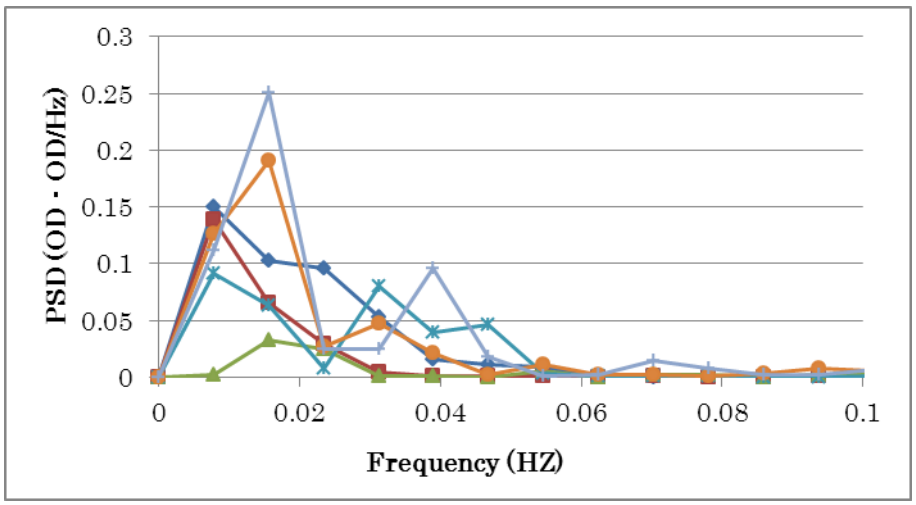
0.04
0.06
0.08
0.1
Frequency (Hz)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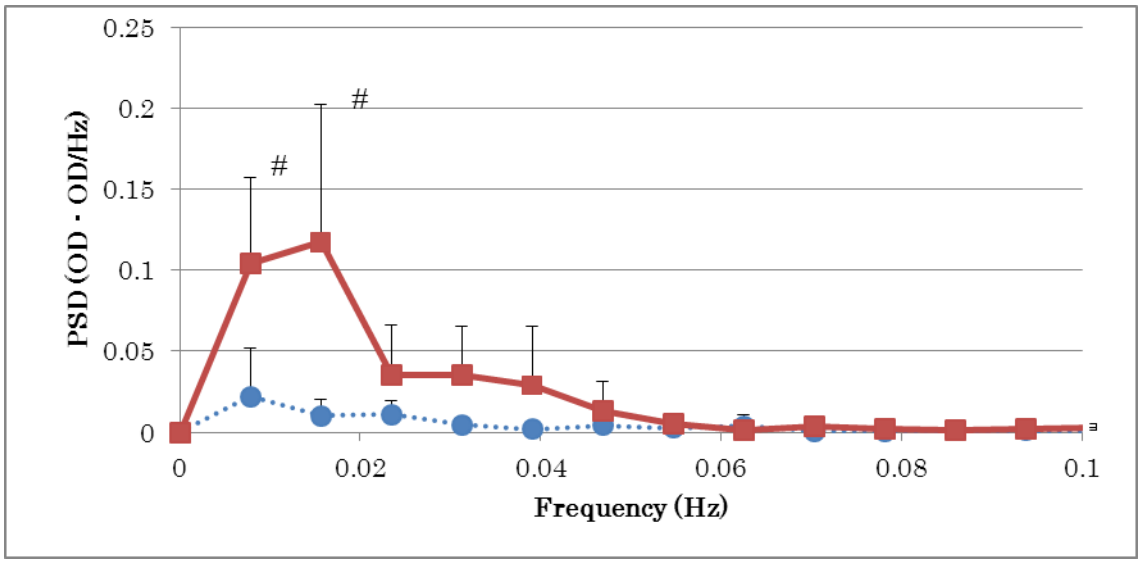
Frequency (HZ)


PSD (OD - OD/Hz)
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0.15
0.1
0.05
0
#
#
0
0.02
0.04
0.06
0.08
0.1
Frequency (Hz)

Fig. 4