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**Muscle pump in the vastus lateralis in the supine position in  
light prolonged exercise**

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Running head: Muscle pump in prolonged exercise

## **Abstract**

**Aim.** The purpose of this study was to examine whether the muscle pump in the supine position is attenuated during light prolonged exercise.

**Methods.** After rest for 5 min, constant-load exercise with 50% of peak oxygen uptake ( $\dot{V}O_2$ ) determined by incremental exercises in the supine position was performed for 60 min with a pedaling rate of 60 rpm. Total hemoglobin and myoglobin (total Hb) in the vastus lateralis was determined by using a near-infrared spectroscopy (NIRS) system. The instrument was operating at 2 Hz.  $\dot{V}O_2$ , heart rate (HR), mean blood pressure (MBP) and muscle deep temperature ( $T_m$ ) were measured in the constant-load exercise.

**Results.** After an increase at the onset of exercise,  $\dot{V}O_2$  showed a steady state, HR showed a significant gradual increase and MBP significantly decreased. After an increase until 20 min of exercise,  $T_m$  showed a steady state. Level of total Hb increased until 20 min and showed a steady state in all subjects. Average  $T_m$  was significantly related to average total Hb ( $r=0.978$ ). Total Hb oscillated, but its oscillation occasionally disappeared. Peak amplitude of oscillation in total Hb for 30 sec after the start of exercise was significantly higher than that for 1 min before the end of exercise.

**Conclusion.** The results suggest that the muscle pump operates in light exercise but is attenuated in the vastus lateralis in the supine position at the late phase of prolonged exercise.

**Key words:** muscle pump-total hemoglobin-venous vessel compliance-supine position.

## Introduction

There is still a debate whether the muscle pump operates well or not in exercise<sup>1</sup>. In e prolonged exercise, it has been reported that the muscle pump is not an important factor for the development of cardiovascular drift<sup>2</sup>, while the muscle pump may operate well when cardiac frequency is equivalent to muscle contraction frequency<sup>3</sup>. It has been suggested that the muscle pump is less important in facilitating venous return and vagal resumption in the supine position than in the upright position<sup>4</sup>. However, those studies did not provide direct evidence of operation of the muscle pump in humans, especially in the supine position. The recent development of near-infrared spectroscopy has made it possible to observe the muscle pump as change in total hemoglobin in the sitting position on a cycle ergometer<sup>5</sup>.

The muscle pump can induce change in blood pressure in venous vessels in the active muscle ( $\Delta P_{mp}$ ). The sum of blood pressure produced by the muscle pump and blood pressure produced by the pooling of blood in venous vessels ( $P_v$ ) can induce the venous return. Since  $P_v$  at rest is higher in the upright position due to the effect of gravity than in the supine position, the difference between blood pressure in the right atrium and that in venous vessels is smaller in the supine position than that in the upright position. Therefore, relative contribution of  $\Delta P_{mp}$  to  $P_v$  should be greater in the supine position than in the upright position.

It has been reported that the level of total hemoglobin is increased at the late phase of prolonged exercise<sup>2, 3</sup>. This means that venous blood is pooled in venous vessels in the muscle. Since the compliance of venous vessels is known to be increased by an increase in temperature<sup>6,7</sup>, the absolute value of blood volume in venous vessels ( $V_v$ ) is increased at the late phase of prolonged exercise due to an increase in muscle temperature in the case of a constant  $P_v$  value. The compliance of venous vessels ( $C$ ) is accurately defined as a function of changed blood volume ( $\Delta V$ ) and changed blood pressure ( $\Delta P$ ). That is,  $C = \Delta V / \Delta P$ . If blood volume changed by the muscle pump ( $\Delta V_{mp}$ ), which is reflected by the change in total hemoglobin, is maintained in the case of an increase in compliance of venous vessels during prolonged exercise,  $\Delta P_{mp}$  is thought to be decreased due to an increase in the compliance. If  $P_v$  is assumed to be constant during prolonged exercise,  $\Delta P_{mp} / P_v$  should be decreased due to an increase in the compliance. This suggests that  $\Delta P_{mp}$  becomes relatively weak in the contribution of  $\Delta P_{mp}$  to  $P_v$  during prolonged exercise.

Therefore, the purpose of the present study was to examine whether the muscle pump is attenuated in the supine position during light prolonged exercise.

## Materials and Methods

### A. Subjects

Eight healthy male volunteers participated in the present study. Their age, height and body weight were  $20 \pm 1.2$  yrs,  $173 \pm 5.8$  cm and  $65 \pm 8.1$  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  $\dot{V}O_2$ ) in the supine position. The power output was set at 20 watts for 1 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 50% peak  $\dot{V}O_2$ . Each subject rested for 5 min in the supine position and performed constant exercise for 60 min with a pedaling rate of 60 rpm.

### C. Measurements

Oxygenated hemoglobin and myoglobin ( $HbO_2$ ) 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  $1.5 \text{ cm}^8$ . The instrument was operating at 2 Hz. This sampling frequency is maximal in the present NIRS system. NIRS is absorbed by hemoglobin and myoglobin. Form changes in the optical densities ( $\Delta OD$ ), changes in oxy-hemoglobin ( $HbO_2$ ) and deoxy-hemoglobin (DHb) were calculated by following equations.

$$\Delta HbO_2 = \Delta OD(840nm) - 0.66\Delta OD(760nm)$$

$$\Delta DHb = -0.59\Delta OD(840nm) + 0.80\Delta OD(760nm)$$

$\Delta$  total hemoglobin (total Hb) is the sum of  $\Delta HbO_2$  and  $\Delta DHb$ . Since total Hb is a relative value, it cannot be used for comparison of total Hb levels between different persons or between different regions of the body. However, data can be used for elucidating the time trend.

We obtained the maximal and minimal values in each oscillation in total Hb for

the first 1 min and last 1 min of prolonged exercise. The difference in maximal and minimal value was defined as amplitude. The peak value in the obtained amplitudes was selected. The average of maximal and minimal values was defined as level of total Hb.

Deep temperature was measured using Core Temp Monitor (CM-210, Termo, Japan). 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 10 min and at the first 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<sup>-1</sup> of ventilation. Fractions of O<sub>2</sub> and CO<sub>2</sub> 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 (O<sub>2</sub>: 15.0%, CO<sub>2</sub>: 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.

#### *E. Statistics*

Data are expressed as means  $\pm$  standard deviation (SD). Dunnett's test was used to determine the significance in differences between the value at rest, at 3 min or at 10 min and values at other times in exercise. Significant level was set below 0.05. The strength of the relationship between dependent and independent values was expressed by the single correlation coefficient of Pearson.

### **Results**

As shown in Figure 1,  $\dot{V}O_2$  significantly increased and then showed a steady state. MBP significantly increased and showed a significant decrease at 60 min compared to the value at 10 min in prolonged exercise. HR significantly increased from the onset of exercise and showed significant gradual increases after 20 min compared to the value at 3 min in prolonged exercise.

Total Hb in a typical subject is shown in Figure 2. Total Hb increased until 20 min after the start of exercise and then showed a steady state (upper panel). This tendency was observed in all subjects. After a sudden decrease, HbO<sub>2</sub> increased until 20 min and then showed a steady state. The steady state level was below zero. This tendency was similar to that of total Hb. DHb suddenly increased and showed a steady state until the end of exercise. Total Hb decreased at the starting point and showed oscillations with 2 Hz (lower panel). This tendency was observed in all subjects, but the

magnitude of the initial decrease in total Hb was very small in two subjects.

Kinetics of muscle temperature in prolonged exercise and the relationship between average muscle temperature and average total Hb obtained at the time of measurement of muscle temperature are shown in Figure 3. Muscle temperature significantly increased from the resting value until 20 min and then showed a steady state. Average  $T_m$  was significantly related to average total Hb ( $r=0.978$ )

As shown in Figure 4, there were still oscillations in total Hb. Total Hb drifted up and down with each pedaling. However, the amplitude of oscillation sometimes decreased and the oscillation occasionally disappeared. Level of total Hb at the early phase was lower than that at the late phase of prolonged exercise

As shown in Table 1, peak amplitude of oscillation in total Hb for 30 sec after the start of exercise was significantly higher than that for 1 min before the end of exercise. Level of total Hb for 30 sec before the start of exercise was significantly lower than that for 1 min before the end of exercise.

### **Discussion**

In the supine position, venous blood pooling could not be a zero level and total Hb therefore decreased and oscillated after the first pedaling. The oscillation sometimes disappeared because sample frequency of NIRS was 2 Hz and pedaling rate was 2 Hz. When the pedaling is in the sampling duration, total Hb is decreased. When relaxation of muscle is in the next sampling duration, total Hb is increased. This leads to oscillation of total Hb. However, when the pedaling is in the middle of two samplings, total Hb in the two samplings can be the same due to the counteractions in total Hb during sampling. Therefore, disappearance of oscillation does not mean that there is no muscle pump. The muscle pump operates during prolonged exercise.

The first decrease in total Hb was observed in the first pedaling. This is a very important result. If blood in the muscle was squeezed out of venous vessels in the muscle by the muscle pump, the blood that had been out squeezed would have been increased in the large venous vessels out of the muscle. In this case, total Hb should be increased. The present result suggests that oscillation of total Hb may occur in venous vessels in the muscle. In fact, it is thought that most of the Hb signal comes from small vessels, because large arteries and veins have large heme concentrations that absorb all of the light<sup>8</sup>. This means there is no detectable signal change in larger vessels.

Level of total Hb was increased at the late phase of prolonged exercise. This means that venous blood is pooled in venous vessels in the muscle. As mentioned in Introduction, blood pooling in the muscle occurs in the late phase of prolonged exercise.

Compliance of venous vessels ( $C = \Delta V / \Delta P$ ) can also be increased in relation to an increase in muscle temperature<sup>6,7</sup>. Due to an increase in the compliance, it is thought that  $\Delta P_{mp}$  should be decreased even in the case of constant  $\Delta V_{mp}$  during prolonged exercise. When blood pressure in venous blood in the muscle ( $P_v$ ) is assumed to be constant,  $\Delta P_v / P_v$  (rate of contribution of the muscle pump to total blood pressure in venous blood in the muscle) should be decreased during prolonged exercise.

MBP was decreased at the late phase of prolonged exercise. This means that peripheral resistance is decreased<sup>9</sup> since cardiac output has been reported to be constant during prolonged exercise. The difference between MBP and  $P_v$  would be decreased, but blood flow in the muscle should maintain due to the decrease in peripheral resistance<sup>9</sup>. In this case,  $P_v$  might be remained constant during prolonged exercise.

Peak amplitude of oscillation for 30 sec after the start of exercise was significantly higher than that for 1 min before the end of exercise. This indicates that  $\Delta V_{mp}$  should be small in the late phase of exercise. In the case of increase in compliance and decrease in  $\Delta V_{mp}$ ,  $\Delta P_{mp}$  becomes considerably smaller than that in the case of constant  $\Delta V_{mp}$  as mentioned above. This means that the muscle pump is ineffective at the late phase of prolonged exercise. Therefore, the ineffective muscle pump could result in a decrease in stroke volume and consequently increase in HR.

Intramuscular pressure (IMP) is known to be 55 mmHg at 70% maximal voluntary contraction (MVC) and about 30 mmHg at 25% MVC<sup>10</sup>. Force in dynamic cycling performed at peak  $\dot{V}O_2$  level corresponds to 50% of MVC<sup>11</sup>. In the present study, since 50% level of peak  $\dot{V}O_2$  was used, the estimated MVC at this work load corresponds to about 25% MVC. Therefore, IMP could be about 30 mmHg. This is a very low level compared with the levels in soleus and tibialis anterior muscles during walking and running<sup>12</sup>. Therefore, not all of the pooled blood could be pumped by muscle contraction in the vastus lateralis. Furthermore, since the effect of the muscle pump may be depending on IMP in muscle groups working at the same work rate, each muscle group could have different effect of muscle pump.

Attention must be given to the effect of skin blood flow on total Hb determined by NIRS. Skin blood flow increases in prolonged exercise<sup>13</sup>. It is known that heating induces an increase of total Hb<sup>14</sup>. However, as mentioned above, it seems that compliance of venous vessels in the muscle is increased, and peak amplitude of oscillation in total Hb due to the muscle pump was not increased despite an increase in the compliance. If all of the pooled blood had been squeezed out of venous vessels in the muscle during muscle contraction, more blood would have been supplied to the muscle in the late phase of prolonged exercise due to an increase in compliance during

relaxation of the muscle. However, this was not the case in the present study. This suggests that there is pooling of blood in the muscle during exercise. Therefore, even if there is an effect of an increase in skin blood flow on level of total Hb during prolonged exercise, it would be partial.

There are other limitations in the present study. Sampling frequency was 2 Hz. This is maximal sampling frequency in the present instrument. This may cause incorrect estimation of peak amplitude. Although a recently developed instrument uses the spatially resolved spectroscopy (SRS)<sup>15</sup> method, we used the modified Beer-Lambert (MBL) method in the present experiment. The relative value was given by the MBL method. Therefore, intra-individual comparison cannot be carried out. However, data obtained in continuous measurement can be compared.

### **Conclusion**

The results suggest that the muscle pump operates in light exercise but is attenuated in the vastus lateralis in the supine position at the late phase of prolonged exercise.

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## Legends of figures

Figure 1. Oxygen uptake ( $\dot{V}O_2$ ), mean blood pressure (MBP) and heart rate (HR) in prolonged exercise. Arrow shows significant difference from the resting value. \*: significant difference from the value at 10 min (MBP) or 3 min (HR) in exercise.

Figure 2. Total Hb, HbO<sub>2</sub> and DHb level in prolonged exercise in a typical subject (upper panel). Oscillations of total Hb for 30 sec after the start of exercise in the same typical subject for whom results are shown in the upper panel (lower panel).

Figure 3. Muscle deep temperature (T<sub>m</sub>) in prolonged exercise (upper panel). Relationship between average T<sub>m</sub> and average total Hb (lower panel). Arrow shows significant difference from the resting value.

Figure 4. Oscillations of total Hb for 1 min before the end of exercise in four subjects.

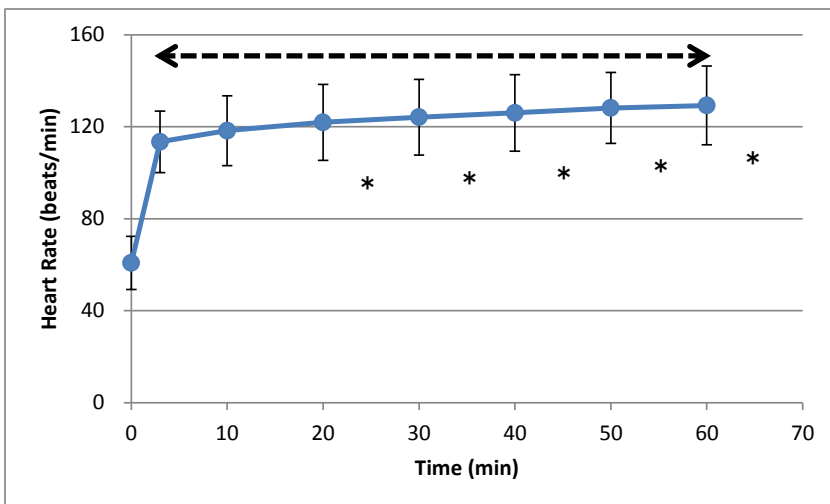
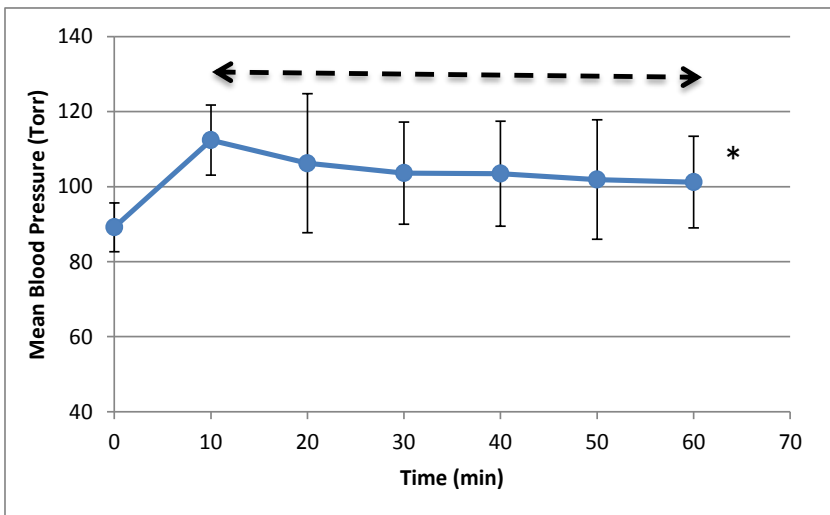
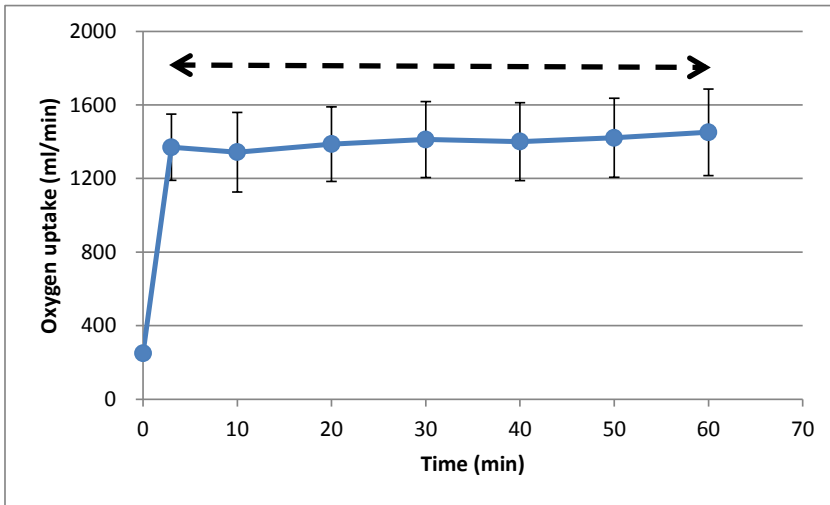


Fig. 1.

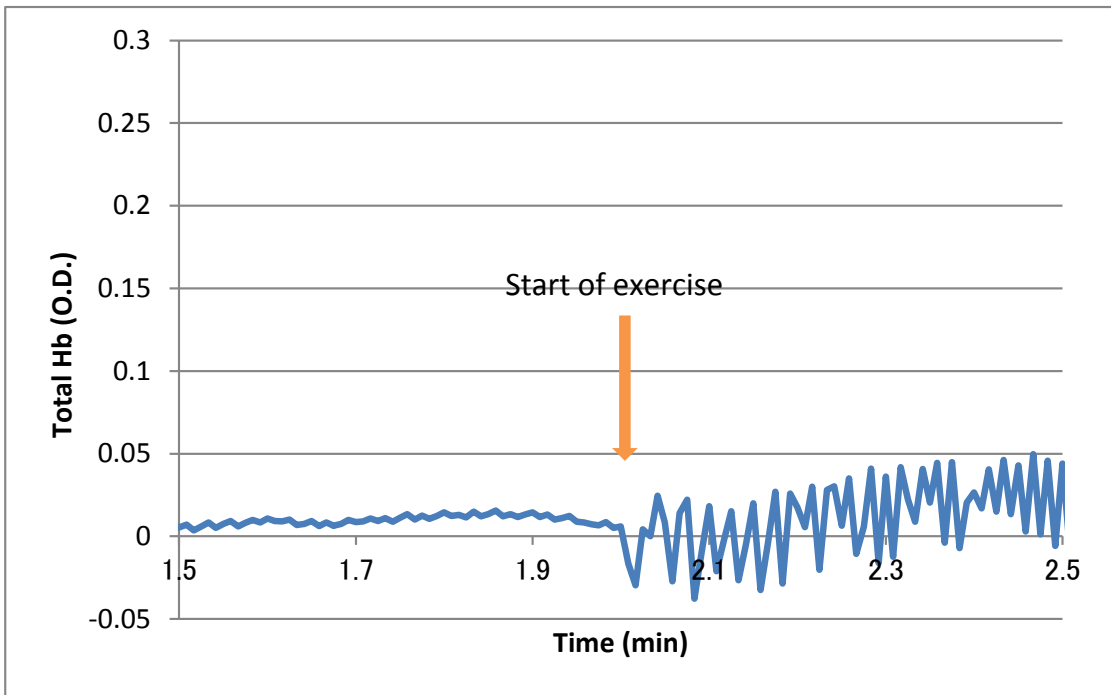
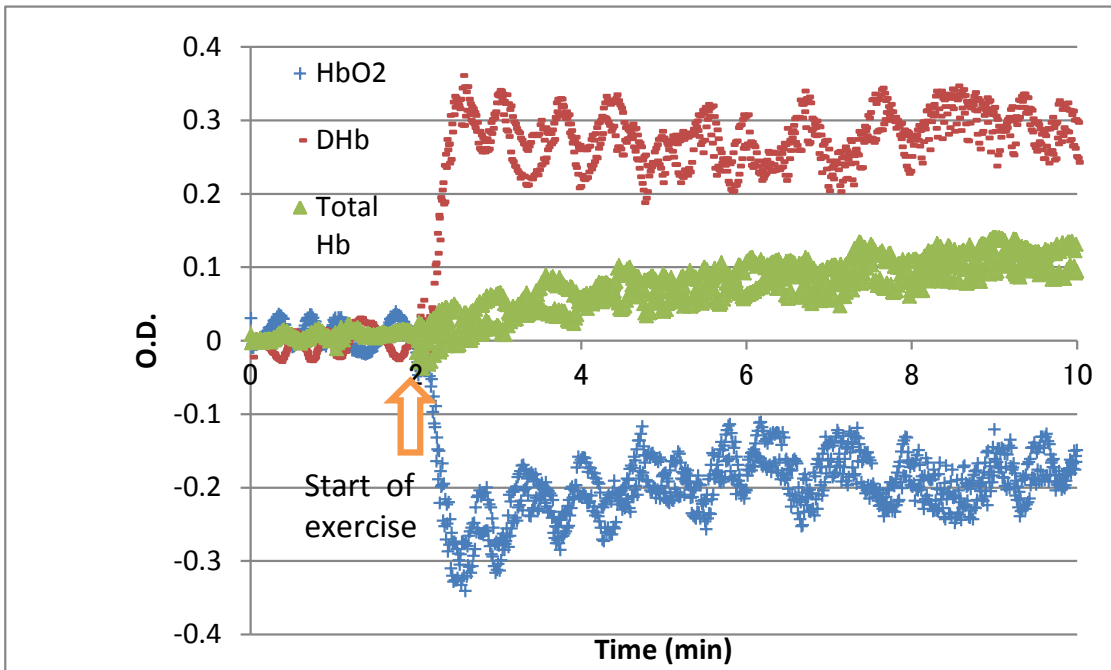


Fig. 2.

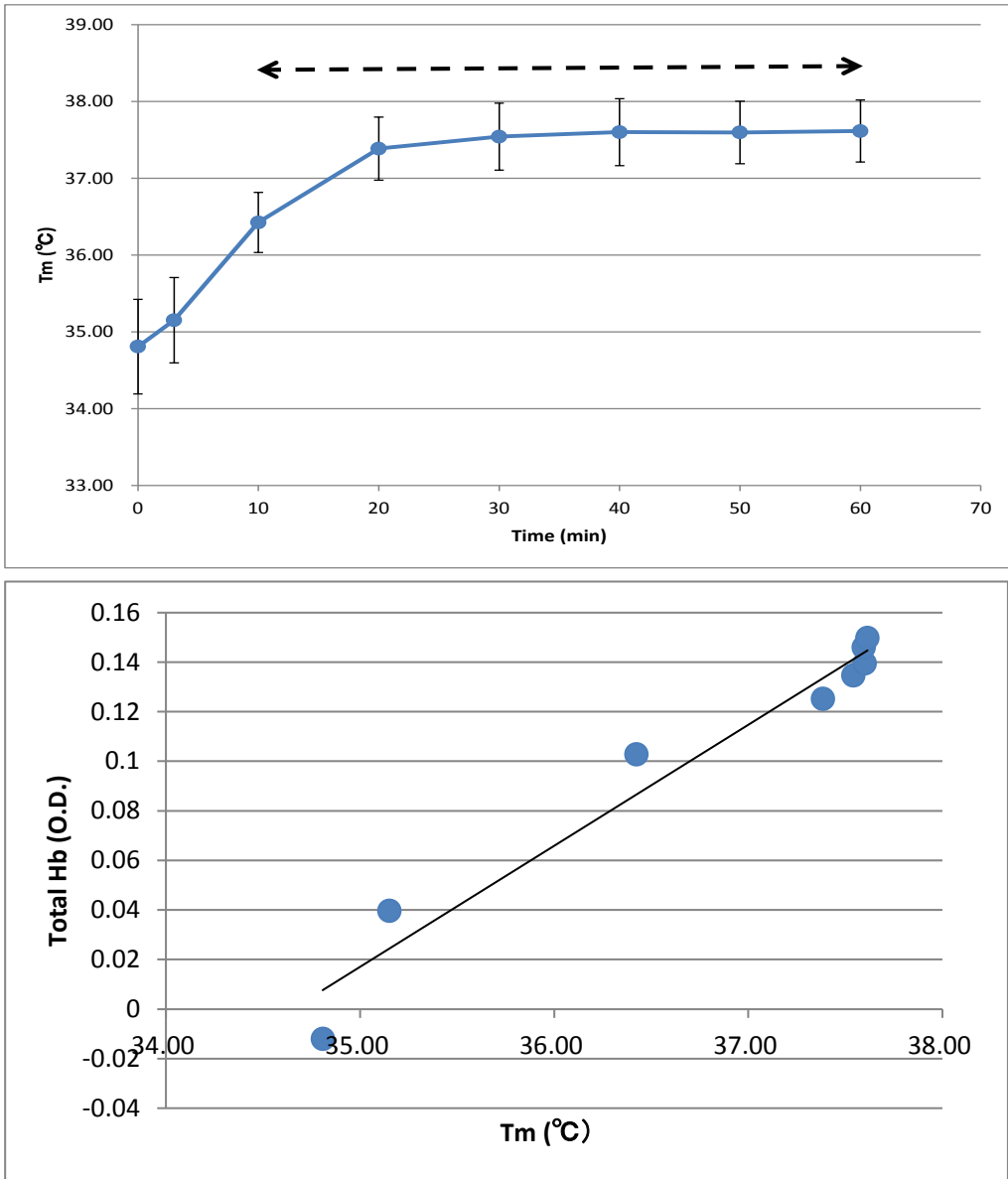


Fig. 3.

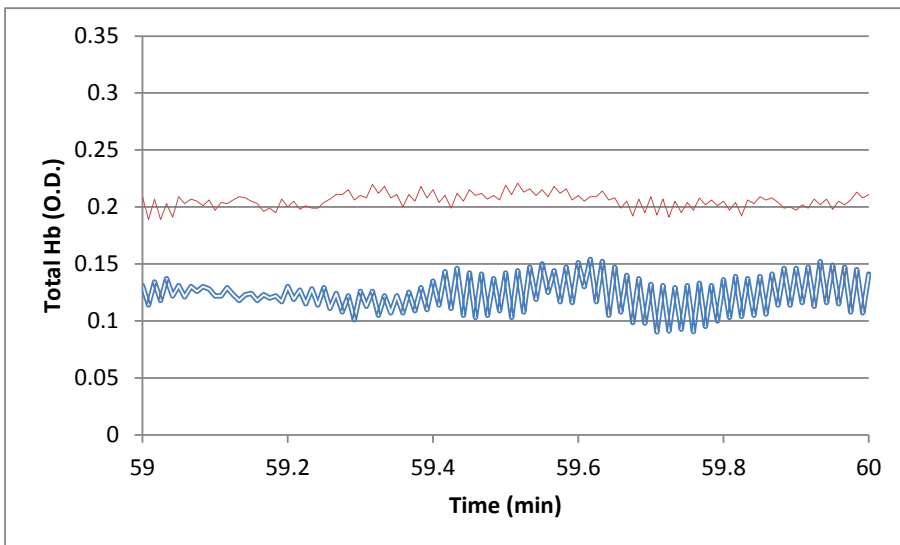
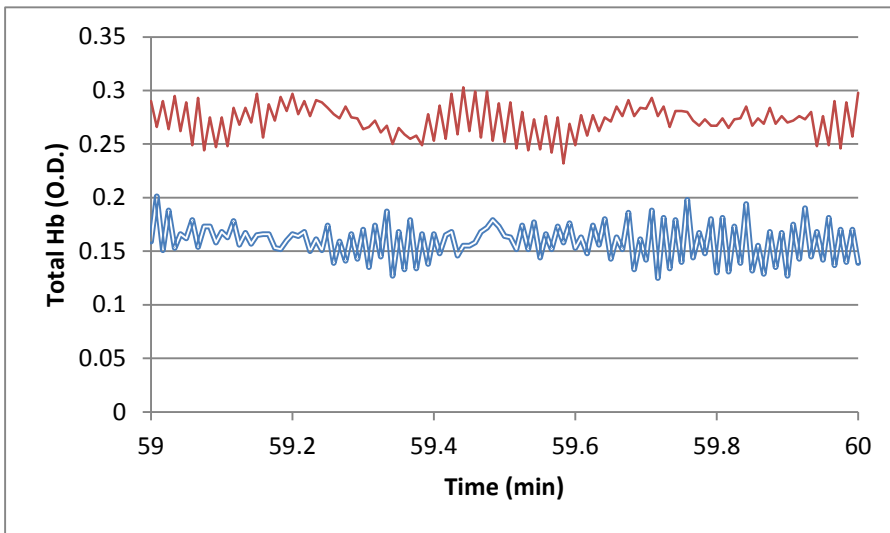


Fig. 4.