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Relationship Between Maximal Oxygen Uptake and Oxygenation Level in Inactive Muscle at Exhaustion in Incremental Exercise in Humans

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Summary

The aim of the present study was to determine whether the oxygenation level in an inactive muscle during an incremental exercise test, determined by near-infrared spectroscopy, influences the maximal oxygen uptake ($\dot{V}O_{2\max}$). The oxygenation level at the onset of incremental exercise was higher than that at rest and started to decrease at a high power output. A minimal level was observed at exhaustion during incremental exercise. $\dot{V}O_2$ increased linearly after some delay, and the rate of increase in $\dot{V}O_2$ was greater at a higher power output. Heart rate increased linearly after the time delay, and the rate of increase in heart rate did not change. There was a significant correlation between $\dot{V}O_{2\max}$ and oxygenation level in inactive muscle at exhaustion ($r = -0.89$). We therefore concluded that the oxygenation level in inactive muscle at exhaustion during incremental exercise is associated with an individual difference in $\dot{V}O_{2\max}$.

Key words

Oxygenation • Inactive muscle • Incremental exercise • Maximal oxygen uptake

Introduction

Oxygen uptake ($\dot{V}O_2$) increases with an increase in power output, but there is an upper limit of the increase in $\dot{V}O_2$. This is maximal $\dot{V}O_2$ ($\dot{V}O_{2\max}$). It has been shown that $\dot{V}O_{2\max}$ changes when oxygen delivery is altered by administration of a β -blocker (Tesch 1985), by hypoxia due to carbon monoxide (Ekblom *et al.* 1975) and by blood doping (Ekblom *et al.* 1976, Gledhill 1982, Spriet *et al.* 1986) and that the increase in $\dot{V}O_{2\max}$ with training results primarily from an increase in maximal cardiac output ($Q_{\max}$) (Saltin *et al.* 1968). It has also been shown that small muscle mass has an extremely high capacity

for oxygen consumption (Saltin 1985). These results suggest that oxygen delivery primarily limits $\dot{V}O_{2\max}$ (Bassett and Howley 2000).

Cardiac output (Q) increases with an increase in power output (Peltonen *et al.* 2001). $\dot{V}O_2$ linearly increases with higher power output at low exercise intensity. When the $\dot{V}O_2$ -power output relation is extrapolated into higher power output, measured $\dot{V}O_2$ is higher than $\dot{V}O_2$ estimated from the relation (Zoladz *et al.* 1998). The higher $\dot{V}O_2$ is called excessive $\dot{V}O_2$ or slow component of $\dot{V}O_2$. Ventilation also linearly increases and then exponentially increases with higher power output. Hyperventilation requires excessive $\dot{V}O_2$ (Aaron *et al.*

1992). However, excessive $\dot{V}O_2$ is derived not only from hyperventilation but also from active muscle (Poole *et al.* 1991). At high exercise intensity, it has been shown that muscle sympathetic nervous tone is activated (Victor *et al.* 1987, Saito *et al.* 1999). This results in a decrease in blood flow in inactive muscle (Bevegard and Shepherd 1966). The decrease in blood flow in inactive muscle may facilitate an increase in blood flow in active muscle. It means that blood flow into active muscle may be affected by attenuation of blood flow in inactive muscle as well as by an increase in cardiac output.

Oxygen supply to active muscle is mainly due to an increase in cardiac output at low exercise intensity and attenuation of oxygen supply into inactive muscle would be added during incremental exercise at high intensity. Finally, maximal oxygen supply to active muscle is associated with both Q_{max} and attenuated value of oxygen supply to inactive muscle. This should determine $\dot{V}O_{2max}$. However, the decrease in oxygen supply to inactive muscle has not been examined as a limiting factor of $\dot{V}O_{2max}$, probably because it is difficult to measure blood flow even at inactive muscle exhaustion by a non-invasive technique.

Near-infrared spectroscopy (NIRS) is a new method by which oxygenation level in the tissue can be determined. We have examined oxygenation level in inactive muscle during exercise using NIRS (Ogata *et al.* 2002). We found that the oxygenation level did not change at low exercise intensity but decreased at high exercise intensity after about two minutes of exercise. Since the oxygenation level is determined by a balance of oxygen supply and since oxygen consumption can be assumed to be constant in inactive muscle, the decrease in oxygenation level in inactive muscle can be assumed to be due to the decrease in oxygen supply. Moreover, it has been pointed out that oxygenation kinetics in inactive muscle during exercise is similar to the characteristics of activation of sympathetic nervous system due to exercise. This suggests that attenuation of oxygen supply is due to vasoconstriction in inactive muscle during exercise.

Therefore, in the present study the effect of oxygenation level in inactive muscle determined by NIRS on $\dot{V}O_{2max}$ was examined during incremental exercise.

Methods

Subjects

Seven healthy male volunteers participated in the present study. Their age, height and body weight were

27.0±4.0 years, 170±6.1 cm and 63±5.6 kg, respectively. Consent for participation in the study was obtained from all subjects after informing them about the purpose of the experiment, the procedure and possible risks. The study was approved by the local ethics committee.

Experimental protocol

An electrically braked cycle ergometer (Combi 232C, Japan) controlled by a computer was used in the experiment. After a 5-min rest period, the power output was increased by 25 watts per minute until the subject was unable to maintain a pedaling rate of 60 rpm. After the incremental exercise test, the subject remained in a resting state for 40 min.

Measurements

In the present study, the NIRS signal was measured from the right upper arm (flexor carpi radialis muscle) every 5 seconds, while the blood pressure was measured from the left upper arm at 2-min intervals. For these measurements, the subject was asked to keep his arms at rest as much as possible on a table adjusted to the heart level throughout the resting, exercise and recovery periods.

Oxygenated and deoxygenated hemoglobin concentrations (HbO_2) were measured using NIRS (HEO200N, Omuron, 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 the detector. Dual-wavelength light (760 and 840 nm) emitted from the light source penetrates the 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 (MacCully and Hamaoka 2000). After a recovery period, a pneumatic cuff (MT-720, Mizuho, Japan) was fixed to the right upper arm, and arterial occlusion above 300 mm Hg was carried out for more than 10 min. During arterial occlusion, HbO_2 decreased abruptly and then decreased gradually in all subjects.

Systolic and diastolic blood pressures were determined non-invasively by synchronizing Korotkov sound with heart beat by an electro-sphygmomanometer (STPS-680, Nippon Colin, Japan). Mean blood pressure (MBP) was calculated as diastolic pressure plus one third of the pulse pressure.

Ventilation and gas exchange responses were measured by an on-line computerized breath-by-breath method (AE-280S, Minato Medical Science, Japan). A two-liter syringe was used to calibrate the system,

which was linear throughout a range of 0-600 l·min⁻¹ of ventilation. Fractions of O₂ and CO₂ 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. The oxygen uptake and carbon dioxide output were measured at 15-s intervals. Heart rate was recorded using a heart rate monitor installed in the respiratory gas analyzer.

Determination of Vo₂max and ventilatory threshold

The peak value of Vo₂ obtained in incremental-load exercise was certified to be Vo₂max when more than two of the following three criteria were met: 1) no increase or reduction in Vo₂ despite the increase in work load, 2) peak heart rate of more than 90 % of maximal heart rate (HRmax) predicted by age (predicted HRmax = 220 – age), and 3) respiratory gas exchange ratio exceeding 1.15 (Howley *et al.* 1995, Pimentel *et al.* 2003). Ventilatory threshold was determined by V-slope method (Beaver *et al.* 1986).

Assessment of oxygenation level

Oxygenated (HbO₂) and deoxygenated hemoglobin (Hb) absorbs light corresponding to their concentrations. The light which is not absorbed is scattered. This scattered light can be detected. In the body, the absolute value of scattered light cannot be determined but the changing rate can be determined. The changing rate is dependent on concentration of HbO₂ and Hb according to the following equation:

$$\Delta OD_{840} = k_1 \cdot \Delta[HbO_2] + k_1' \cdot \Delta[Hb], \quad (1)$$

where ΔOD_{840} indicates a change in optical density at 840 nm, k is a constant value dependent on absorption and scattering of light and $\Delta[HbO_2]$ and $\Delta[Hb]$ denote changes in the concentrations of HbO₂ and Hb, respectively. In order to solve Eq. (1), another equation is needed. Therefore, two wavelength lights are required.

$$\Delta OD_{760} = k_2 \cdot \Delta[HbO_2] + k_2' \cdot \Delta[Hb], \quad (2)$$

where k_2 and k_2' are assumed to be constant. From Eqs. (1) and (2), HbO₂ and Hb can be obtained.

$$\Delta[HbO_2] = K \cdot [\Delta OD_{840} - (k_1'/k_2') \cdot \Delta OD_{760}], \quad (3)$$

$$\Delta[Hb] = K \cdot (k_2/k_2') \cdot [(k_1/k_2) \cdot \Delta OD_{760} - \Delta OD_{840}] \quad (4)$$

where K is equal to $(1/k_2)/(k_1/k_2 - k_1'/k_2')$.

On the basis of *in vitro* examinations, Shiga *et al.* (1997) proposed the constant values of k as following

$$\Delta HbO_2 = K \cdot [\Delta OD_{840} - 0.66 \cdot \Delta OD_{760}] \quad (5)$$

$$\Delta Hb = K \cdot [0.80 \cdot \Delta OD_{760} - 0.59 \cdot \Delta OD_{840}]. \quad (6)$$

Total hemoglobin can be defined as the sum of Eqs. (5) and (6).

$$\Delta HbO_2 + \Delta Hb = K \cdot [0.21 \cdot \Delta OD_{840} + 0.14 \cdot \Delta OD_{760}]. \quad (7)$$

The oxygen saturation can be defined by dividing Eq. (5) by total hemoglobin. However, since value is a changing rate and the optimal density is set by zero level at starting point, oxygen saturation is not mathematically determined. Therefore, the difference between Eqs. (5) and (6) has been used for oxygen saturation (Grassi *et al.* 1999)

$$\Delta HbO_2 - \Delta Hb = K \cdot [0.41 \cdot \Delta OD_{840} - 1.46 \cdot \Delta OD_{760}]. \quad (8)$$

However, Eqs. (5) and (8) are incompatible, and Eq. (8) lost the theoretical background of Eq. (1). Moreover, since the value obtained by Eq. (8) is a relative value, it cannot be used for individual comparison.

Eq. (5) shows that changes in oxygenation can be basically expressed by the difference between OD840 and OD760. Indeed the difference is reported to be related to oxygen saturation in the skeletal muscle (Chance *et al.* 1992, Mancini *et al.* 1994). However, as this value is not an absolute value, it cannot be used for individual comparison. Therefore, the muscle oxygenation level was normalized using the arterial occlusion method. In this study, the muscle oxygenation level at rest was defined as 100 % and the lowest value recorded during arterial occlusion was defined as 0 %. This method has been reported to be reproducible (Higuchi *et al.* 2002).

Statistical analysis

The strength of the relationship between dependent and independent values was expressed by the single correlation coefficient of Pearson. Data are expressed as means $\pm$ standard deviation (S.D.). Repeated ANOVA was used to determine the differences among values. If the F ratio was significant, Fisher's PLSD was used as a post-hoc test. The level of significance was set at $P < 0.05$.

Results

Oxygenation levels quantitatively showed individual differences. In all subjects, oxygenation level increased and decreased at a low work rate, but the degree of the increase was different. A rapid decrease was observed at a higher work rate in four subjects, and a gradual decrease was observed in the other subjects. Figure 1 shows the average changes in oxygenation level. The oxygenation level significantly increased at 25 watts and started to decrease significantly from 156 watts in comparison with the value at 25 watts.

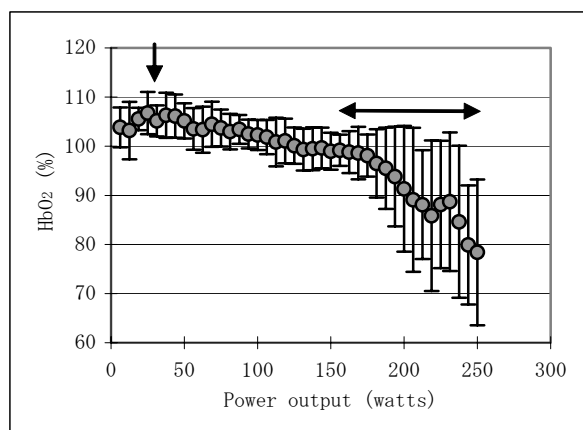


Fig. 1. Kinetics of oxygenation level in inactive muscle during incremental exercise test. Arrows show significant differences between the values at rest and at 25 watts and between the values at 25 watts and at the higher power output.

Figure 2 shows the average changes in heart rate (HR) and $\dot{V}O_2$. HR decreased and increased linearly while $\dot{V}O_2$ increased linearly after the time delay and the rate of increase was changed at a high power output. Figure 3 shows the difference between $\dot{V}O_2$ measured and $\dot{V}O_2$ estimated from the relation between power output and $\dot{V}O_2$ from 50 watts to ventilatory threshold. Below 50 watts, $\dot{V}O_2$ deviated from a linear line of the relation. The power output at ventilatory threshold was 150 ± 23.6 watts. $\Delta \dot{V}O_2$ significantly increased from 162.5 watts. At 250 watts, $\Delta \dot{V}O_2$ was 200 ml/min, while oxygenation level had decreased by 20 %.

Figure 4 shows the relationship between $\dot{V}O_{2\max}$ and oxygenation level at exhaustion. $\dot{V}O_{2\max}$ was 2.72 ± 0.27 l/min. There was a significant correlation ($r = -0.89$). However, a significant correlation was not obtained between $\dot{V}O_{2\max}$ per body weight and ventilatory threshold. There was also no significant relationship between $\dot{V}O_{2\max}$ and power output at exhaustion.

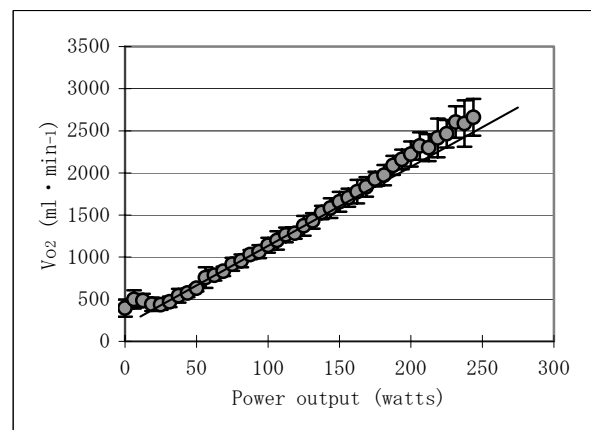
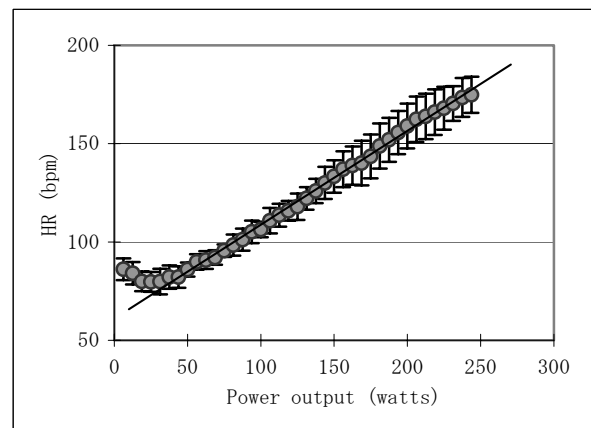


Fig. 2. Kinetics of oxygen uptake ($\dot{V}O_2$) and heart rate (HR) during incremental exercise test as a function of power output.

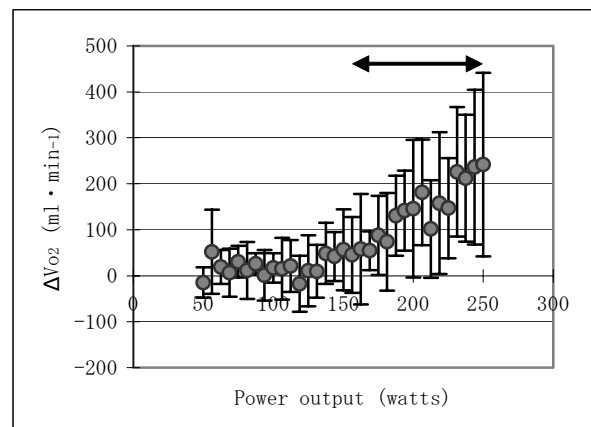


Fig. 3. Difference in measured values of oxygen uptake ($\dot{V}O_2$) and values estimated by power output- $\dot{V}O_2$ relationship obtained below the ventilatory threshold as a function of power output. The arrow shows a significant difference from the values at lower power output.

Figure 5 shows changes in mean blood pressure. There was no significant difference between MBP at rest and at 50 watts. However, MBP significantly increased from the resting level thereafter.

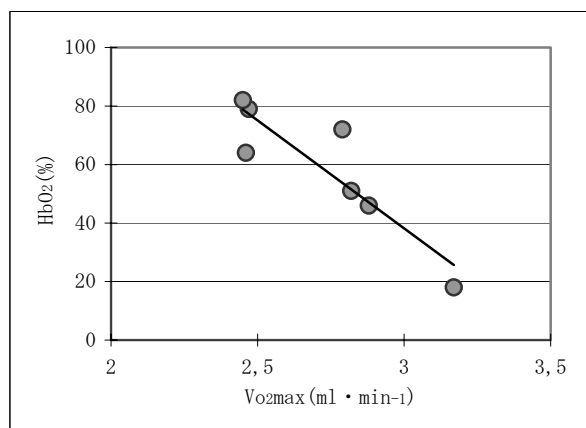


Fig. 4. Relationship between oxygenation level (HbO₂) at exhaustion and maximal oxygen uptake (Vo₂max) obtained during an incremental exercise test.

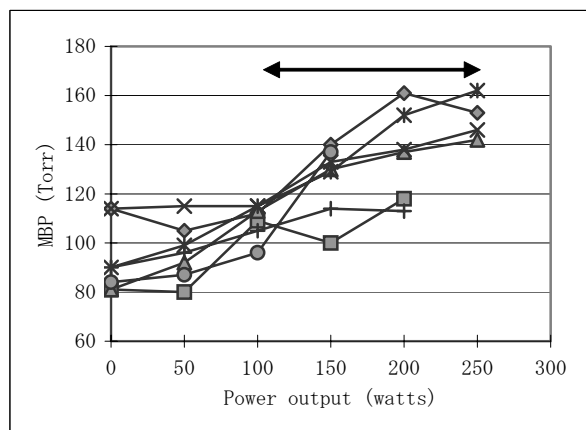


Fig. 5. Mean blood pressure (MBP) during incremental exercise test and at rest were shown as a function of power output. Arrow shows a significant difference from resting value.

Discussion

There was no significant difference between MBP at 50 watts and that at rest. Since cardiac output increases soon after the start of exercise, this unchanged MBP suggests vasodilatation in active muscles. After ventilatory threshold (150 watts), the oxygenation level decreased. Since oxygenation level is a balance between oxygen supply and since oxygen consumption is assumed to be constant in inactive muscle, the decrease in oxygen level reflects a decrease in oxygen supply (Ogata *et al.* 2002). At this power output, mean BP showed a higher level. Therefore, vasoconstriction may occur in the inactive muscle.

It was thought that Vo₂ increased linearly after a time delay in incremental exercise tests (Whipp *et al.*

1981), but a recent study has indicated that Vo₂ kinetics is a non-linear system (Swanson and Hughson 1988). Practically, it is known that by using the regression line obtained Vo₂-power output relation at below ventilatory threshold, the increased values in Vo₂ at a higher work rate can be estimated (Zoladz *et al.* 1998). The estimated excessive Vo₂ is called the slow component.

The slow component has extensively been studied. There are currently two hypotheses (Tschakovsky and Hughson 1999). One is that metabolic inertia is a controller of Vo₂, and the other is that oxygen availability limits Vo₂. The present study suggests that the reduction in oxygen supply to inactive muscle causes an increase in oxygen supply to active muscles so that Vo₂ increases at a higher power output. However, the present study does not provide direct evidence of an increase in the oxygen supply in active muscles.

It has recently been reported that Vo₂max determined in incremental exercise tests with different increasing rates of exercise intensity is not affected by the duration of exercise, but Qmax decreases in the exercise with long duration (6-12 min) in comparison with short duration (6 min) (McCole *et al.* 2001). The reduction of Qmax may be due to the difference in recruited muscle mass (Lepretre *et al.* 2004). Because slower incremental exercise test, a subject finished at relatively lower exercise intensity and at low exercise intensity recruited muscle mass is considered to be smaller. In this case, attenuation of oxygen supply to inactive muscle may compensate the reduction of Qmax to maintain Vo₂max.

Vo₂max is related to the oxygenation level at exhaustion. The relationship between Vo₂max and oxygenation level may be derived from sympathetic nerve activity. Vasoconstriction in inactive muscle during exercise is associated with muscle sympathetic nerve activity. When the level of this activity is high, the oxygenation level becomes low. If this high activity accompanies sympathetic nerve activity to the heart muscle, Qmax could be high. Therefore, low oxygenation level at exhaustion would indicate high attenuation of blood flow in inactive muscle and high Qmax, suggesting high oxygen supply to active muscle.

We therefore conclude that the individual difference in reduction of oxygen supply to inactive muscle during incremental exercise causes an individual difference in Vo₂max.

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Reprint requests

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