

Original Investigation

Vastus Lateralis Oxygenation During Prolonged Cycling in Healthy Males

Kotaro Kawaguchi¹, Yukiko Hayashi², Kiyokazu Sekikawa¹, Mitsuru Tabusadani³

Tsutomu Inamizu¹, Kiyoshi Onari⁴ and Yagesh Bhambhani⁵

1) Division of Physical Therapy and Occupational Therapy Sciences, Graduate School of Health Sciences. Hiroshima University, Japan

2) Mori Clinic, Hiroshima, Japan

3) Yurino Hospital, Nagasaki, Japan

4) Department of Health and Sports Sciences, Fukuyama Heisei University, Japan

5) Faculty of Rehabilitation Medicine, University of Alberta, Canada

Address for correspondence:

Dr. Yagesh Bhambhani

Room 373 Corbett Hall, Faculty of Rehabilitation Medicine

University of Alberta, Edmonton, Canada T6G 2G4

e-mail: yagesh.bhambhani@ualberta.ca

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Abstract

This study examined the relationship between the acute cardiorespiratory and muscle oxygenation/blood volume changes during prolonged exercise. Eight healthy male volunteers (mean $\dot{V}O_{2\max} = 41.6 \pm 2.4$ ml/kg/min) performed 60 minutes submaximal cycling at 50% of $\dot{V}O_{2\max}$. Oxygen uptake ($\dot{V}O_2$) was measured by indirect spirometry, cardiac output (CO) was estimated via Portapres, and right vastus lateralis oxyhemoglobin/myoglobin (OxyHb/Mb), deoxyhemoglobin/myoglobin (DeoxyHb/Mb) and total hemoglobin/myoglobin (Total Hb/Mb) were recorded using near infrared spectroscopy. After 40 minutes of exercise, there was a significant increase in $\dot{V}O_2$ due to a significantly higher $(a - v)O_{2\text{diff}}$. After 30 mins of exercise CO was unchanged, but there was a significant decrease in stroke volume and a proportionate increase in heart rate indicating the occurrence of cardiovascular drift. During the first few minutes of exercise, there was a decline in OxyHb/Mb and Total Hb/Mb while DeoxyHb/Mb remained unchanged. Thereafter OxyHb/Mb and Total Hb/Mb increased systematically until the termination of exercise while DeoxyHb/Mb declined. After 40 minutes of exercise, these changes were significantly different from the baselines values. There were no significant correlations between the changes in the NIRS variables and systemic $\dot{V}O_2$ or mixed $(a - v)O_{2\text{diff}}$ during exercise. These results suggest that factors other than localized changes in muscle oxygenation and blood volume account for the increased $\dot{V}O_2$ during prolonged submaximal exercise.

Key words: near infrared spectroscopy, cardiovascular drift, systemic oxygen consumption.

Introduction

Near infrared spectroscopy (NIRS) is a non-invasive optical technique that has been used to continuously measure oxygen uptake kinetics of the vastus lateralis in vivo. NIRS is based on the differential absorption properties of hemoglobin (Hb) and myoglobin (Mb) in the near infrared range of 600 to 1000 nm. At a wavelength of 760 nm, Hb and Mb occur in the deoxygenated form (DeoxyHb/Mb), whereas at 850 nm these chromophores occur in the oxygenated state (OxyHb/Mb). The difference in absorbency between these two wavelengths reflects the balance between oxygen supply and utilization at the level of the small blood vessels (ie. the arterioles, capillaries and venules) and provides an index of the relative change in muscle oxygenation. The sum of the absorbency signals is an index of the localized change in blood volume at the measurement site (Chance et al., 1992; Mancini et al., 1994). Numerous studies have documented the changes in muscle oxygenation and blood volume measured by NIRS during incremental (Belardinelli et al., 1995a; Bhambhani et al., 1997; Grassi et al., 1999; Kawaguchi et al., 2001) and steady state cycling exercise at various intensities (Belardinelli et al., 1995b; Bhambhani et al., 1998; Chuang et al., 2002). It is generally accepted that the localized changes in muscle oxygenation and blood volume measured by NIRS can accurately track alterations in whole body oxygen uptake at intensities below and above the lactate (ventilatory) threshold.

The cardiorespiratory and metabolic responses during prolonged cycling have been well documented. Research has demonstrated that during prolonged submaximal exercise lasting beyond 10 to 15 minutes, the systemic oxygen uptake ($\dot{V}O_2$) demonstrates a small but significant increase, while the cardiac output (CO) usually remains unchanged (Cheatham et al. 2000; Coyle

and Gonzales-Alonso, 2001). However, the manner in which the CO is maintained during prolonged exercise is altered. Typically, there is a steady decline in the stroke volume (SV) and a proportionate increase in the heart rate (HR) in order to maintain CO. The physiological factors implicated in this phenomenon, usually referred to as the *cardiovascular drift*, are unclear. Initial research (Rowell, 1974) suggested that the reduction in SV was due to the redistribution of blood flow to the skin in order to stimulate sweating and maintain thermal balance. It was postulated that the increased skin blood flow reduced the central blood volume, thereby decreasing ventricular filling and SV. However, subsequent research (Coyle, 1998; Coyle and Gonzales-Alonso, 2001) indicated that approximately 50% of the decline in SV observed during prolonged exercise was due to hyperthermia while the remainder was due to dehydration.

The alterations in the metabolic and cardiovascular responses during prolonged exercise raise two important questions. Firstly, if systemic $\dot{V}O_2$ increases during prolonged exercise, is this due to an increase in localized oxygen extraction? Secondly, if CO is maintained but a greater proportion of the blood is directed to the periphery, does this decrease blood volume and oxygen availability in the exercising muscle? To date, only one investigation has examined the muscle oxygenation responses during prolonged cycling exercise. Neary et al.(2002) examined these changes during a 20 kilometer cycling time trial lasting between 20 min to 30 min in well trained subjects and reported a systematic decline in this variable without any significant alteration in systemic $\dot{V}O_2$ until the termination of the test. However, they did not measure the CO nor did they examine the alterations in blood volume during the time trial, thereby making it difficult to fully interpret their findings. The purpose of this study, therefore, was to examine the relationship

between the acute cardiorespiratory, metabolic and vastus lateralis oxygenation/blood volume changes during 60 minutes of cycling exercise in healthy subjects. It was hypothesized that beyond 20 minutes of exercise there would be a significant: (1) decline in SV which would be accompanied by a proportionate increase in HR (ie. evidence of cardiovascular drift), (2) decrease in muscle oxygenation and blood volume measured by NIRS, and (3) increase in systemic $\dot{V}O_2$ which would be correlated with greater muscle deoxygenation (ie. greater extraction) during exercise.

Methods

1. Subjects

Eight healthy male volunteers participated in this study. Although the subjects exercised approximately 3 times a week, none of them were endurance-trained athletes. The details of the study were explained to the subjects and measurements were performed after written informed consent was obtained. The Ethics committee of Hiroshima University, Faculty of Medicine, approved this study. Each subject completed the following two exercise testing sessions within a one-week period. Both the tests were conducted under similar laboratory conditions at a room temperature of 25°C and 54% relative humidity. The physical and physiological characteristics of the subjects are summarized in Table 1.

2. Session One: Determination of Maximal Oxygen Uptake

Upon reporting to the testing laboratory, the height and body mass of the subject was recorded using standardized procedures. Thereafter, the subject sat on the cycle ergometer (232CXL) for approximately five minutes for preparation of the cardiorespiratory measurements and to establish a resting baseline. Exercise was initiated by pedaling at 50 rpm at an initial power output of 30W for one minute as a warm up. The power output was then increased to 50W for three minutes and this increment was followed in a stepwise fashion until voluntary fatigue, or two of the following criteria for $\dot{V}O_{2max}$ were attained (ACSM, 1994): (1) leveling of in the $\dot{V}O_2$ with increasing work rate, (2) age predicted maximal HR (220 - age yrs), and (3) a respiratory exchange ratio > 1.10 . Cardiorespiratory measurements were recorded continuously during these tests using a computerized Aeromonitor (AE-280s, Minato Medical Science, Japan) in the breath-by-breath mode. The instrument was calibrated using precision gases (16% oxygen, 4% carbon dioxide, balance nitrogen) before and after each test to ensure accuracy of the data. The pneumotach was calibrated using a 2 L syringe prior to each measurement. The HR was recorded continuously during the test with an ECG monitor DS-3140 (Model DS-3140, Fukuda Denshi Co., Japan). These data were averaged every 20 seconds and the highest values used for analysis.

3. Session Two: Prolonged Exercise Test

The prolonged exercise test was performed using the same cycle ergometer as the stepwise incremental test. The subject rested on the cycle ergometer for approximately five minutes to facilitate preparation of the physiological and NIRS measurements and achieve a resting baseline condition. This was followed by a one-minute warm-up of unloaded pedaling and

60 minutes of cycling at 50% of the subject's $\dot{V}O_{2\max}$. The power output at which this intensity was attained was determined by linear regression analysis between the power output and $\dot{V}O_2$ for each subject based on the stepwise incremental exercise test. During this test, the cardiorespiratory responses were measured using the same instrumentation described earlier.

(4) Measurement of Cardiac Output during Prolonged Exercise

During the prolonged exercise test, the CO was measured using a non-invasive monitor (Portapres Model 2, TNO-TPD Biochemical Instrumentation, Amsterdam, Netherlands) according to the Modelflow method (Wesseling et al., 1993). The validity of this technique against the Doppler method and its repeatability during cycle exercise at intensities above and below the VT has been demonstrated (Sugawara et al., 2003). The procedure for this measurement conducted in our laboratory has been previously described (Hayashi et al., 2002). Briefly, a cuff was placed on the middle finger of the right hand that was placed on a hand rest at the heart level. The subject was asked not to extend the cuffed finger and to maintain an upright posture during exercise so as to ensure accuracy of the data. A height correction factor was used to compensate for hydrostatic changes in blood pressure in the finger. Beat by beat blood pressure waveforms and flow measurements were recorded at each 10 min segment of the prolonged exercise test to calculate the CO. From these values, the SV was calculated as the quotient between CO and HR. The mixed arterio-venous oxygen difference $[(a-v)O_{2\text{diff}}]$ was calculated as the quotient between the absolute $\dot{V}O_2$ and CO.

(5) Measurement of Muscle Oxygenation During Prolonged Exercise

During the prolonged exercise test, NIRS measurements from the right vastus lateralis muscle were recorded continuously using a laser tissue blood oxygenation monitor (BOM-L1TR, Omegawave Inc., Japan). This instrument uses 3 laser-diodes (780, 810 and 830 nm) to determine changes in the oxygenation status of Hb and Mb. The distance between the incident point and the detector was 30 mm, with a resulting penetration depth of approximately 15 mm. The NIRS probe was placed along the vertical axis of the thigh, approximately 12 to 15 cm from the knee joint. The probe was secured in place with a tape and rubber plate so that blood flow to the muscle was not occluded. The data were collected on a personal computer (Power Book G4, Apple computer) at a sampling frequency of 10 Hz via an A/D transducer (Mac Lab, AD Instrument Inc.).

The changes in absorption at each wavelength were converted into concentration changes of OxyHb/Mb and DeoxyHb/Mb using the modified Beer-Lambert law in which a differential path-length factor (DPF) was incorporated to correct for the scattering of photons in the tissue. In the current study a DPF of 4.0 was used for all the subjects because direct measurement of the individual DPF was not possible using continuous-wave spectrophotometry (Matcher et al., 1995). The absorption coefficient of Hb at each wavelength was based on the data reported by Matcher et al. (1995). The absorption changes were converted into concentration changes using the following algorithm:

- $\text{Oxy Hb} = 9.09 \cdot \text{Ln} \cdot (\text{Ib} / \text{Ibo} - \text{Ic} / \text{Ico}) - 0.54 \cdot \text{Ln} \cdot (\text{Ia} / \text{Iao} - \text{Ib} / \text{Ibo})$
- $\text{Deoxy Hb} = 5.73 \cdot \text{Ln} \cdot (\text{Ib} / \text{Ibo} - \text{Ic} / \text{Ico}) - 3.61 \cdot \text{Ln} \cdot (\text{Ia} / \text{Iao} - \text{Ib} / \text{Ibo})$

In the above equations, \ln is the natural logarithm, I_a and I_{a0} , I_b and I_{b0} , and I_c and I_{c0} are the intensities of detected light and incident light from the 780, 810 and 830 wave-lengths, respectively. Individual values were calculated and expressed as $\mu\text{mol/L}$. The integrated and mean values for each minute were calculated using analytical software (Chart v3.6.1/s) and subjected to statistical analysis.

6. Statistical Analysis

For each subject, the mean values of the cardiorespiratory and NIRS responses at each 10 min segment of the 60 min test were calculated. The delta values of these responses with respect to the 10 minute values were calculated and expressed as a percentage change ($\Delta\% \dot{V}O_2$, $\Delta\% CO$, $\Delta\% SV$, $\Delta\% \dot{HR}$, $\Delta\% \text{OxyHb}$, $\Delta\% \text{DeoxyHb}$, and $\Delta\% \text{TotalHb}$). These serial values were compared using a one-way repeated measures analysis of variance (ANOVA) with time as the repeated measure. A two-way repeated measures ANOVA was performed to evaluate the differences between $\dot{V}O_2$ and NIRS data during the prolonged exercise test. When a significant main effect was detected without any interaction, further examination using a one-way repeated measures ANOVA and Fisher's PLSD post hoc test was conducted. Linear regression analysis was used to examine the relationships between the changes in $\dot{V}O_2$, $(a - v)O_{2\text{diff}}$ and NIRS variables obtained during the prolonged exercise test. The level of significance was set at $p < 0.05$ for all tests. The statistical analyses were performed using Stat View 5.0J software (SAS Institute Inc.).

Results

1. Acute Cardiorespiratory Responses during Prolonged Exercise

All the subjects successfully completed the 60 minutes of cycle exercise at 50% of $\dot{V}O_{2max}$. In each subject, this intensity was below the ventilatory threshold (VT) that was identified by the gas exchange method described by Wasserman et al. (1973). The changes in $\dot{V}O_2$ and mixed $(a - v)O_{2diff}$ during the 60 min of steady state exercise are illustrated in Figure 1. It is evident that during this exercise bout, the $\dot{V}O_2$ remained fairly stable during the first 40 min of exercise and then increased significantly during the final 20 min. The mean increase in systemic $\dot{V}O_2$ at the end of the end of 60 min compared to the 10 min stage was 6.4%. The mixed $(a - v)O_{2diff}$ demonstrated a significant increase during the exercise and reached its peak at 30 min of exercise. Thereafter, it began to decline so that the value at the end of the 60 min exercise period was not significantly different from that observed at the 10 min interval. The cardiovascular responses during the 60 min of exercise are illustrated in Figure 2. All the subjects demonstrated changes that were characteristic of the cardiovascular drift phenomenon. Specifically, the CO remained unchanged, but there was a significant decline in the SV beyond 40 min of exercise when compared to the values during the first 30 min. This was accompanied by a proportionate increase in HR so that the CO was unchanged during the 60 min of exercise.

2. Muscle Oxygenation and Blood Volume Changes during Prolonged Exercise

Of the eight subjects, the NIRS data of one subject was eliminated due to technical problems. Figure 3 demonstrates the typical NIRS trend in a representative subject during the

prolonged exercise test. Generally, the Total Hb/IMb, OxyHb/Mb and DeoxyHb/Mb demonstrated an initial decrease at the onset of exercise, followed by a gradual increase until approximately 10 min of exercise. Thereafter, all three variables attained a temporary steady state. During the last 20 min of exercise, TotalHb/Mb and OxyHb/Mb showed a gradual systematic increase, while DeoxyHb/Mb showed a gradual systematic decrease over the same time period. Statistical analysis of the serial changes in the NIRS responses indicated no significant alterations in $\Delta\%$ OxyHb, $\Delta\%$ DeoxyHb and $\Delta\%$ TotalHb during the 60 min exercise session, as illustrated in Figure 4.

3. Relationship between Systemic VO_2 and NIRS data

Regression analysis was used to examine the individual relationships of the systemic $\dot{V}O_2$ and the mixed $(a - v)O_{2diff}$ with the three NIRS variables, namely, OxyHb/Mb, DeoxyHb/Mb and Total Hb/Mb and. No significant correlations were observed for any of these relationships in these subjects. As well, no significant correlations were observed when the delta values of VO_2 (ie. difference between the values observed at 10 minutes of exercise and each subsequent 10 minute value) were correlated with the delta values of the NIRS variables measured in this study.

Discussion

1. Cardiorespiratory Responses During Prolonged Exercise

In the current study, the systemic $\dot{V}O_2$ demonstrated a small but significant increase during the 60 min exercise bout. The magnitude of this increase was comparable to that reported by Cheatham et al. (2000) and Coyle and Gonzales-Alonzo (2001). Cheatham et al. (2001)

reported a 4.9% increase in the $\dot{V}O_2$ after 40 min of steady state cycle exercise performed below the VT by healthy men. They attributed this small but significant increase in $\dot{V}O_2$ to alterations in motor unit recruitment, enhanced utilization of fat, and an elevated core body temperature. In the current study, the increase in systemic $\dot{V}O_2$ was attained primarily by a significant increase in the $(a - v)O_{2diff}$ (ie. greater oxygen extraction) as the CO was unchanged. The $(a - v)O_{2diff}$ reached its peak at 30 min of exercise and then began to decline. However, the values at 40 min and 50 min were still significantly higher than the value observed at 10 min, while that observed at 60 min was not significantly different. The magnitude of the increase in the $(a - v)O_{2diff}$ at 30 min was consistent with that reported by Cheatham et al. (2000) who reported an increase of 6% in men exercising at an intensity equivalent to the VT. As well, Coyle and Gonzales-Alonso (2001) indicated a mean increase ranging from 6% to 8% after 30 min to 40 min of prolonged exercise. The decline in the $(a - v)O_{2diff}$ during the last 30 minutes of the exercise session in this study suggests that the increase in systemic $\dot{V}O_2$ during this period was attained by a redistribution of localized blood flow as the mean CO was unchanged. The Total Hb/Mb changes measured by NIRS, which reflect the localized blood volume, support these observations (see below).

All the subjects in this study demonstrated the changes that were characteristic of the cardiovascular drift phenomenon during prolonged exercise. This was evident by the fact that although CO remained unchanged during the 60 min of exercise, there was a significant reduction in SV with a concomitant increase in HR during the exercise session. In the current study, the SV declined by 17% while the HR increased by 13% during the 60 min exercise bout. The magnitudes of these changes were consistent with those of previous studies (Coyle and Gonzales-Alonso,

2001; Cheatham et al., 2001; Hiyashi et al. 2001) in exercising men. Cheatham et al. (2001) reported a 11.6% decline in SV from 10 min to 40 min of cycling exercise below the VT with a concomitant 13.6% increase in HR. Similarly, Coyle and Gonzales-Alonso (2001) reported a mean decline of 15 % in the SV after 50 min of exercise which was accompanied by an increase in HR of similar magnitude. These investigators attributed the decline in SV to the interactive effects of an increase in core body temperature, reduction in blood volume and elevated HR.

2. Muscle Oxygenation and Blood Volume Changes during Prolonged Exercise

The NIRS trends in the current study indicated an initial decrease in OxyHb/Mb, DeoxyHb/Mb and Total Hb/Mb during the first few minutes of the prolonged exercise test. Thereafter, the OxyHb/Mb and Total Hb/Mb demonstrated a progressive increase for the remainder of the test duration. During this period, DeoxyHb/Mb showed concomitant changes in the opposite direction. The decrease in deoxyHb was of smaller magnitude than the increase in oxyHb, as is evident from Figures 3 and 4. The fact that OxyHb/Mb increased while DeoxyHb/Mb decreased during prolonged exercise implies that the balance between oxygen supply and delivery at the level of the small blood vessels increased (ie. indirectly suggesting that oxygen extraction decreased) despite an increase in the systemic $\dot{V}O_2$. While these observations may appear contradictory, it should be noted that there was a systematic increase in the TotalHb/Mb (an index of localized blood volume) during the last 20 min of the exercise bout when there was an increase in the systemic $\dot{V}O_2$.

Currently, there are no published reports pertaining to the acute NIRS responses during

prolonged moderate intensity submaximal cycle exercise. Belardinelli et al. (1995) and Bhambhani et al. (1998) reported that the muscle deoxygenation patterns during constant work rate exercise lasting between 4 to 6 minutes were dependent upon the exercise intensity. Both these investigations demonstrated that at intensities below the VT, muscle oxygenation decreased during the first two to three minutes of exercise, and thereafter, there was a plateau or a slight increase for the remainder of the work period. This was in contrast to the trends observed at intensities above the VT where the muscle oxygenation demonstrated a continuous decrease throughout the exercise period. Recently, Chuang et al. (2002) reported that muscle oxygenation showed an initial decrease at the onset of 10 min steady state cycle exercise below the VT, followed by a steady increase until the termination of the test. They attributed the initial decrease to an increase in skin blood flow, as this was obliterated by local heating of the skin to 120°F. Jensen-Urstad et al. (1995) evaluated the changes in biceps brachii oxygenation during 15 min of arm cranking exercise performed at a constant work rate. Their results indicated a rapid initial decrease in oxygenation during the first few minutes of exercise with a steady reversal during the latter phases of the test. Neary et al. (2001) reported that during a 20 kilometer time trial lasting between 20 min to 30 min in well trained cyclists, muscle oxygenation showed a continuous decline throughout the test. These trends were opposite to those observed in the current study and are most likely due to the fact that the subjects were exercising at different exercise intensities. In the current study the subjects exercised at a constant work rate corresponding to 50% VO_{2max} , while in the study by Neary et al. (2001) the subjects performed a racing time trial at approximately 90% to 95% of VO_{2max} . Research (Stringer et al., 1994; Wasserman et al., 1991) has demonstrated that at

intensities above the VT, the accumulation of lactate facilitates the release of oxygen from OxyHb via the Bohr effect, thereby resulting in enhanced deoxygenation measured by NIRS.

Unfortunately, none of the NIRS studies cited above measured the muscle blood volume (ie. Total Hb/Mb) changes during exercise, thereby making it difficult to fully interpret their findings.

Grassi et al. (1999) documented the OxyHb/Mb, DeoxyHb/Mb and TotalHb/Mb changes during incremental exercise in healthy males subjects and observed an increase in DeoxyHb/Mb with a concomitant decrease in the OxyHb/Mb patterns until VO_{2max} was attained. The TotalHb/Mb showed an increase until approximately 50% to 60% of VO_{2max} followed by a leveling off or a decrease until VO_{2max} was attained. The authors suggested that this decline in total blood volume was most likely due to the fact that the relative change in intramuscular pressure exceeded the change in intravascular pressure, thereby resulting a decline in localized blood volume. In the current study, the subjects exercised at 50% of VO_{2max} during the prolonged exercise test, which was below the exercise intensity at which the blood volume began to decline during incremental exercise. It is likely that the steady increase in Total Hb/Mb observed could be due to increases in muscle temperature during the prolonged exercise bout.

In the current study we hypothesized that blood flow to the exercising muscle would be reduced during prolonged exercise because of the cardiovascular drift phenomenon, and as a result, there would be significant declines in the muscle oxygenation and blood volume responses measured by NIRS. The current findings however demonstrated a systematic increase in Total Hb/Mb during exercise (Figure 4), which suggests an increase in localized blood volume directly below the NIRS measurement site. This increase in blood volume was accompanied by a

systematic increase in the OxyHb/Mb while DeoxyHb/Mb showed a slight decrease during the test. The latter findings, which suggest reduced oxygen extraction in the muscle, support the critical oxygen delivery threshold purported by Schumaker and Cain (1987). These researchers hypothesized that oxygen extraction in the muscle was dependent upon blood flow; if blood flow increased then oxygen extraction decreased and vice versa. Hayashi et al. (2002) reported that skin blood flow significantly increased in the first 40 minutes of submaximal cycling exercise. Theoretically, this could have increased the TotalHb/Mb responses observed in this study. Chuang et al. (2002) reported that the contribution of cutaneous blood flow to the overall NIRS response during 6 mins of exercise at an intensity below the VT was quite small (Chuang et al., 2002). Currently, there is no information pertaining to the blood volume responses measured by NIRS during prolonged exercise. The current findings indicate that majority of the increase in Total Hb/Mb occurred during the first 30 minutes of exercise, with a leveling off during the last 30 minutes of the exercise bout (Figure 4). Further research is necessary to verify these blood volume changes measure by NIRS so as to increase our understanding of this area.

3. Relationship between $\dot{V}O_2$ and NIRS data

Previous research has indicated that the kinetics of peripheral muscle oxygenation measured by NIRS reflect systemic $\dot{V}O_2$. Belalardinelli et al. (1995b) documented that the steady increase observed in systemic $\dot{V}O_2$ at exercise intensities above the VT was inversely correlated ($r = -0.69$) with the changes in OxyHb/Mb saturation during 6 min of constant work rate exercise. Kawaguchi et al. (2001) reported that the kinetics of peripheral muscle oxygenation reflected

systemic $\dot{V}O_2$ during incremental cycle exercise in 16 healthy males. They observed significant negative correlations ranging from 0.725 and 0.978 between OxyHb and systemic $\dot{V}O_2$ and positive correlations ranging from 0.893 and 0.986 between DeoxyHb and $\dot{V}O_2$ in their subjects. In the present study, the relationship between the systemic $\dot{V}O_2$ and the NIRS variables was not evident. The significant increase in systemic $\dot{V}O_2$ during prolonged exercise was attributed to a significant increase in the mixed $(a-v)O_{2diff}$, which however, was not significantly related to any of the NIRS variables. These observations are consistent with those of Bhambhani et al. (1999) who reported no significant correlation between the mixed $(a-v)O_{2diff}$ and muscle deoxygenation in healthy males and females performing four minutes of submaximal exercise at intensities below and above the VT. Collectively, the current NIRS findings suggest that the increase in systemic $\dot{V}O_2$ during prolonged exercise is attained by an enhanced localized blood volume as the localized muscle oxygenation decreased at the measurement site.

Davies et al. (1986) reported that both $\dot{V}O_2$ and V_E increased significantly at 110 minutes of a 4-hour exercise protocol. The authors suggested that the increase in V_E reflected an increase in the work of the respiratory muscles that could elevate systemic $\dot{V}O_2$. However, in the present study V_E did not increase significantly during the 60 minutes exercise session. It is possible that the increase in systemic $\dot{V}O_2$ could be due to the elevated HR resulting from the cardiovascular drift during prolonged exercise. Overall, the HR increased by approximately 13% during the cardiovascular drift phase of the exercise bout. Research (Coyle, 1998; Coyle and Gonzales-Alonso, 2001) has indicated that hyperthermia and dehydration contribute equally to the increased HR observed during prolonged exercise. In this study, the subjects were allowed to

consume fluids ad lib, and therefore, it is unlikely that dehydration was a major factor that contributed to the elevated during the prolonged exercise bout.

In summary, the findings of this study demonstrated that during 60 minutes of cycling exercise at 50% $\dot{V}O_{2\max}$ there was a significant increase in systemic $\dot{V}O_2$. This increase was due to an increase in the mixed $(a - v)O_{2\text{diff}}$ as the CO was unchanged during exercise. However, there was evidence of cardiovascular drift, as indicated by a significant increase in HR and a proportionate decrease in SV. The current NIRS findings suggested that the increase in systemic $\dot{V}O_2$ during prolonged exercise was attained by a redistribution of muscle blood volume as the localized muscle oxygenation decreased at the measurement site. Further research is necessary to corroborate these NIRS responses during prolonged exercise.

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Table 1 Physical characteristics of subjects

No.	Age (years)	height (cm)	weight (kg)	BMI	predicted $\dot{V}O_{2\max}$ (ml/min/kg)	peak $\dot{V}O_{2\max}$ (ml/min/kg)	VT point* (% $\dot{V}O_{2\max}$)	established load intensity during prolonged exercise (Watts)
1	20	169	63.8	22.34	45.76	42.93	54	114
2	19	164	57.0	21.19	49.19	42.72	59	113
3	22	171	68.6	23.46	44.11	36.90	58	113
4	21	181	75.2	23.08	42.19	41.42	54	133
5	22	172	68.0	22.99	40.71	39.10	61	100
6	21	179	71.6	22.35	47.77	42.76	59	142
7	21	182	79.4	23.97	53.55	44.29	58	167
8	20	180	72.2	22.28	41.66	42.60	58	126
mean±S.D	20.8 ± 1.0	174.7 ± 6.6	69.5 ± 6.9	22.7 ± 0.9	45.6 ± 4.4	41.6 ± 2.4	57.6 ± 2.4	126 ± 21

* VT was previously obtained by the exercise tolerance test by ramp loading, and the ratio to $\dot{V}O_{2\max}$ obtained by the exercise tolerance test by stepwise incremental protocol (%) was calculated.

Figure legend

Figure 1: Serial changes in oxygen uptake and mixed arterio-venous oxygen difference during 60 minutes of submaximal exercise in healthy males. Data are expressed as means \pm SE. † indicates significantly different from the 10 minute value. * indicates significantly different from the 30 minute value; $p < .05$.

Figure 2: Serial changes in heart rate, stroke volume and cardiac output during 60 minutes of submaximal exercise in healthy males. Data are expressed as means \pm SE. Heart rate and stroke volume showed significant changes that were characteristic of cardiovascular drift. * indicates the value was significantly different from the previous 10 minute value; $p < .05$.

Figure 3: Changes in total hemoglobin (Total Hb), oxyhemoglobin (OxyHb) and deoxyhemoglobin (deoxyHb) during 60 minutes of submaximal exercise in a representative subject. a = rest; b = warm up, c = exercise, d = recovery.

Figure 4: Serial changes in oxygen uptake and NIRS data during 60 minutes of submaximal exercise in healthy males. Data are expressed as means \pm SE. * indicates significantly different from the 10 minute value.

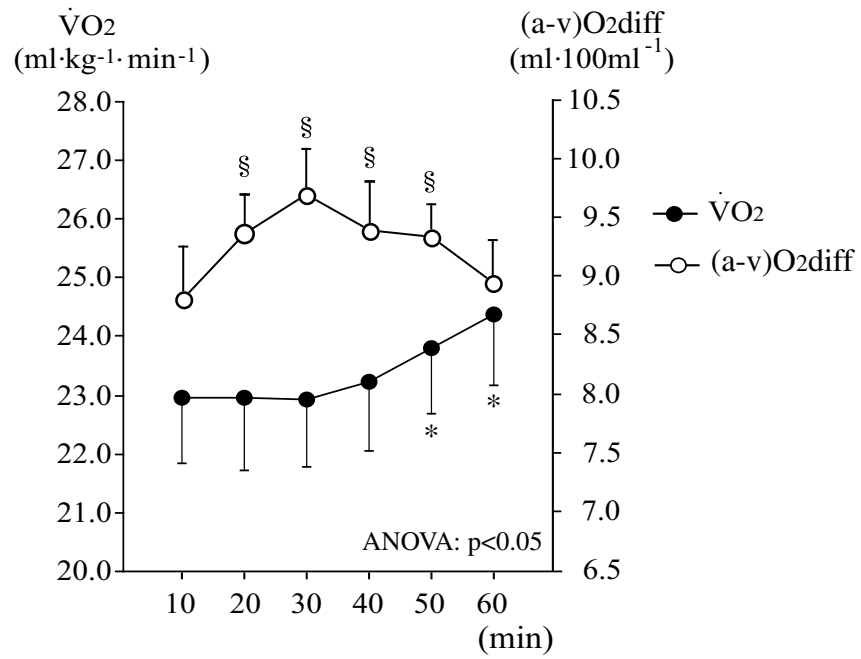


Fig. 1 Serial change in $\dot{V}O_2$ and (a-v)O₂diff during prolonged exercise
 Data express mean \pm S.E.
 §; versus 10 minutes, *; versus 30 minutes

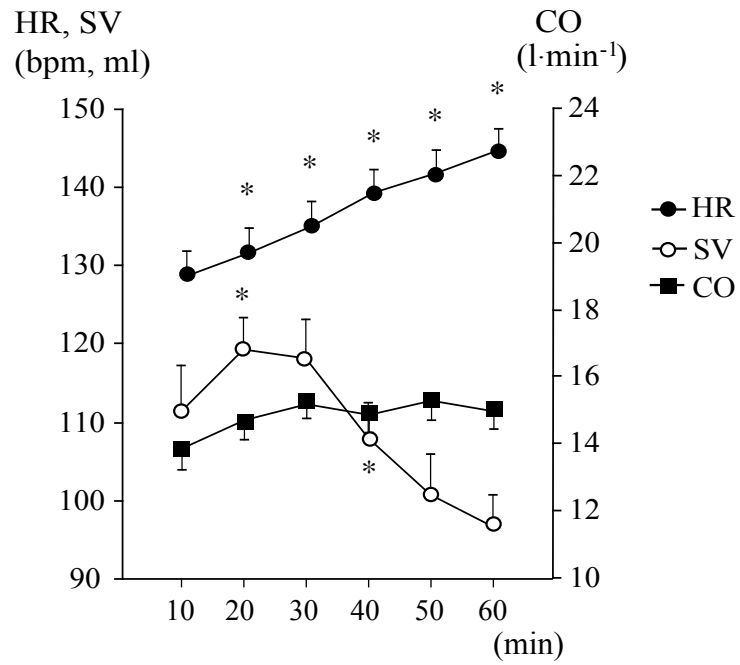


Fig. 2 Serial change in HR, SV, and CO during prolonged exercise
 Data express mean \pm S.E.
 HR and SV showed significant change in time course using one-way repeated ANOVA, $p < 0.05$
 *; Values at this time point are different from previous time point, $p < 0.05$

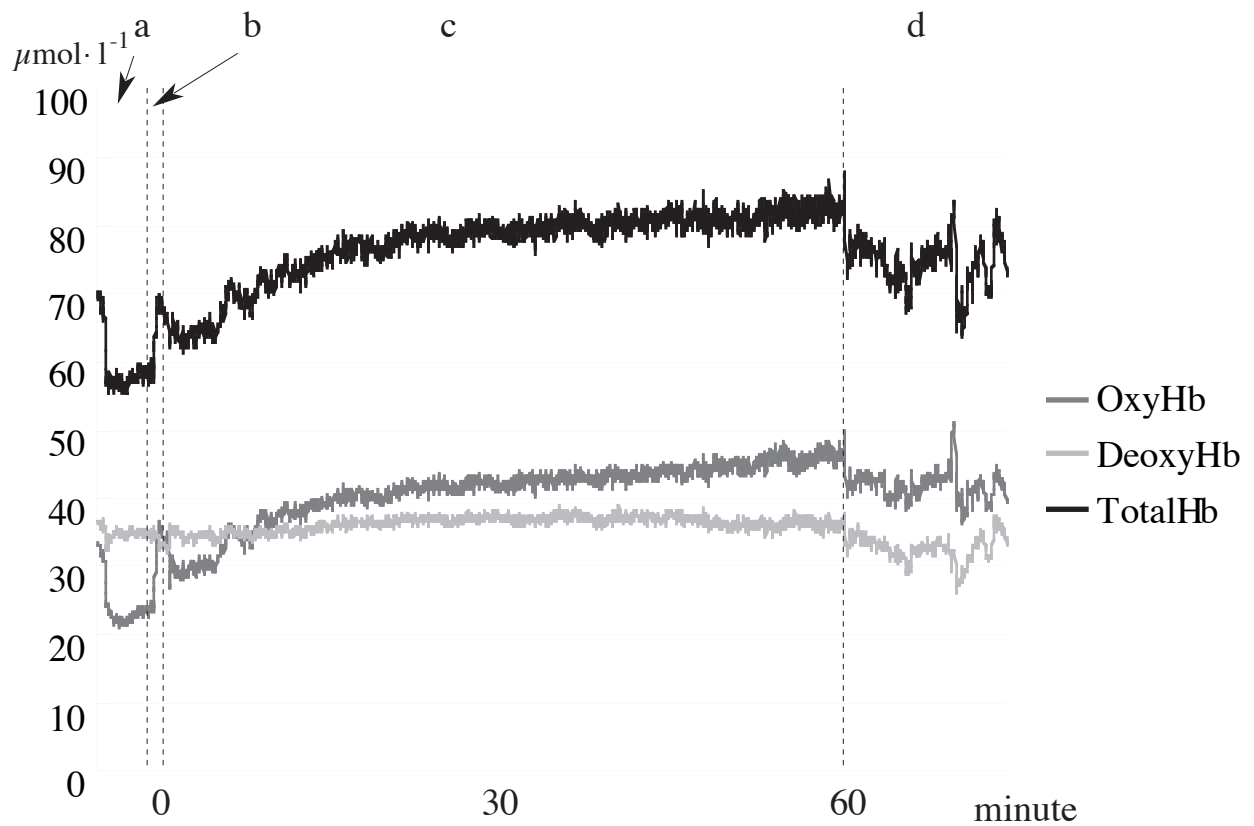


Fig. 3 Typical tracing of change in total hemoglobin (TotalHb), oxyhemoglobin (OxyHb), deoxyhemoglobin (DeoxyHb) during exercise. a; rest, b; warming up, c; exercise, d; recovery

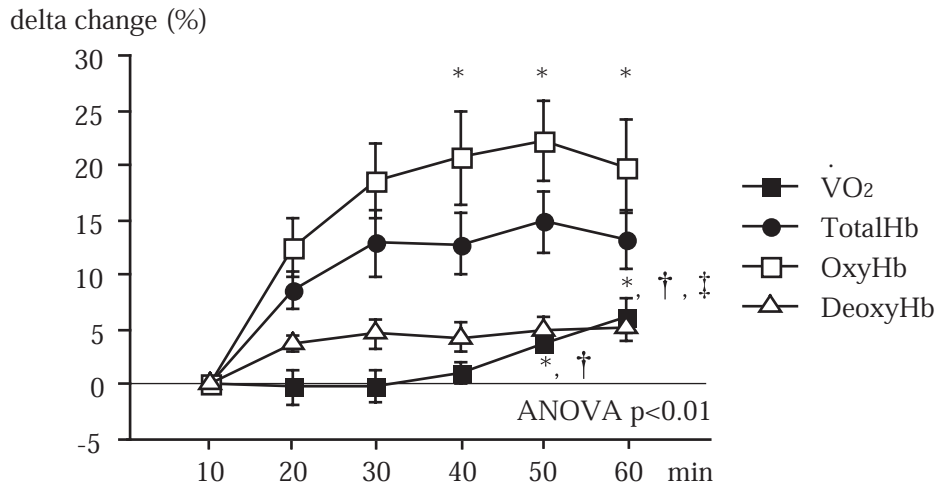


Fig. 4 Serial change in $\dot{V}O_2$ and NIRS data during prolonged exercise.

There were significant differences in serial change of $\dot{V}O_2$ and each NIRS data without any interaction ($p < 0.01$). Data express mean \pm S.E.

*; versus 20 minutes, †; versus 30 minutes, ‡; versus 40 minutes