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Original article

Detection of ventilatory thresholds using near-infrared spectroscopy with a polynomial regression model

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ABSTRACT

Whether near-infrared spectroscopy (NIRS) is a convenient and accurate method of determining first and second ventilatory thresholds (VT_1 and VT_2) using raw data remains unknown. This study investigated the reliability and validity of VT_1 and VT_2 determined by NIRS skeletal muscle hemodynamic raw data via a polynomial regression model. A total of 100 male students were recruited and performed maximal cycling exercises while their cardiopulmonary and NIRS muscle hemodynamic data were measured. The criterion validity of VT_{1VET} and VT_{2VET} were determined using a traditional V-slope and ventilatory efficiency. Statistical significance was set at $\alpha = .05$. There was high reproducibility of VT_{1NIRS} and VT_{2NIRS} determined by a NIRS polynomial regression model during exercise (VT_{1NIRS}, $r = 0.94$; VT_{2NIRS}, $r = 0.93$). There were high correlations of VT_{1VET} vs VT_{1NIRS} ($r = 0.93$, $p < .05$) and VT_{2VET} vs VT_{2NIRS} ($r = 0.94$, $p < .05$). The oxygen consumption (VO_2) between VT_{1VET} and VT_{1NIRS} or VT_{2VET} and VT_{2NIRS} was not significantly different. NIRS raw data are reliable and valid for determining VT_1 and VT_2 in healthy males using a polynomial regression model. Skeletal muscle raw oxygenation and deoxygenation status reflects more realistic causes and timing of VT_1 and VT_2.

1. Introduction

Maximal oxygen consumption (VO_{2max}) is considered the gold standard for evaluating cardiorespiratory function (Mitchell et al., 1958; Saltin and Astrand, 1967). The submaximal anaerobic threshold (AT) and ventilatory threshold (VT) provide a better clinical index of aerobic fitness than VO_{2max}. Previous studies confirmed that the VT was also related to the intensity of aerobic exercise, self-reported activity levels, and training benefits (Tamai et al., 1993; Gaskill et al., 2001). Therefore, developing a convenient and accurate method to determine VT is a significant research issue.

Other methods of estimating the VT have been developed such as tissue deoxygenation (Bhambhani et al., 1997), double product breakpoint (Omiya et al., 2004), non-linear increased EMG activity (Lucia et al., 1997), heart rate deflection (Conconi et al., 1982), and heart rate variability (Cottin et al., 2007). One study indicated that changes in the respiratory frequency were related to the first ventilatory threshold (VT_1) and second ventilatory threshold (VT_2) (Neder and Stein, 2006). The most popular technique is the V-slope method (Beaver et al., 1986), which determines the VT by measuring the non-linear point of increase in the slope of carbon dioxide production (VCO_2) vs oxygen uptake (VO_2) during incremental exercise. The VT reflects lactic acid accumulation during exercise that causes hyperventilation. (Myers and Ashley, 1997).

Near-infrared spectroscopy (NIRS) signals obtained during exercise were confirmed to reflect local tissue oxygen delivery, utilization, and blood flow (Boushel et al., 2001; DeLorey et al., 2003). The primary NIRS parameters include oxygenated hemoglobin (O_2Hb),

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oxygenated myoglobin (O_2Mb), deoxygenated hemoglobin (HHb), and deoxygenated myoglobin (HMb). O_2Hb and O_2Mb were confirmed to reflect tissue O_2 delivery. HHb and HMb were confirmed to reflect tissue O_2 utilization.

The physiological significance of muscle oxygen saturation was demonstrated using different exercise intensities and modes. Previous studies reported that skeletal muscle oxygenation was relatively constant during walking and gradually increased during incremental running (Hiroyuki et al., 2002; Lee et al., 2011). Other studies indicated that muscle oxygenation status follows a sigmoidal profile during incremental cycling (Ferreira et al., 2007; Legrand et al., 2007; Thomas and Stephane, 2008; Racinais et al., 2014; Boone et al., 2015). Studies reported that muscle O_2 delivery (O_2Hb + O_2Mb) increased until moderate intensity and then decreased or was maintained until exercise ended. Other studies found that O_2 delivery decreased from the start to the end of exercise. Further studies indicated that muscle O_2 utilization (HHb + HMb) increased from the beginning to the end of exercise. Other studies found that O_2 utilization increased to high intensity and were then maintained until the end of exercise.

Previous studies indicated that VT_1 and VT_2 could be determined using NIRS based on the breakpoint of skeletal muscle oxygenation and blood flow (deoxy[Hb + Mb] and total hemoglobin [Hb + Mb]) (Boone et al., 2015), muscle oxygenation (O_2Hb) (Legrand et al., 2007; Racinais et al., 2014), the difference between muscle oxygenation and deoxygenation (Δ[O_2HbMb-HHbMb]) (van der Zwaard et al., 2016), and respiratory muscle oxygenation (Moalla et al., 2005). Mathematical models of determining VT_1 and VT_2 include single linear regression (Legrand et al., 2007), double linear regression (Grassi et al., 1999; Moalla et al., 2005; Legrand et al., 2007; Wang et al., 2012), non-linear models (Racinais et al., 2014), and combining linear and polynomial regression (van der Zwaard et al., 2016). Integrating previous studies, the choice of parameters for determining VT_1 and VT_2 were muscle oxygenation (O_2Hb + O_2Mb) or deoxygenation (HHb + HMb). The muscle oxygenation and deoxygenation data in most mathematical models are polynomial patterns (sigmoidal profile).

Although NIRS was confirmed as a non-invasive method to reflect oxygen delivery, utilization, and blood flow during exercise, whether oxygenation and deoxygenation NIRS signals can be used to determine VT_1 and VT_2 requires further clarification. Establishing mathematical models using raw data can reduce data conversion errors. Polynomial regression model verification is also used as a reference for future development of NIRS to automatically determine VT_1 and VT_2. This study investigated the reliability and validity of VT_1 and VT_2 determined via NIRS oxygenation and deoxygenation raw data using a polynomial regression model. This study's hypotheses were that VT_1 and VT_2 determined using raw NIRS data and a polynomial regression model would have good reliability and viability.

2. Materials and methods

2.1. Subjects

This study was approved by the ethics committee of Chang Gung Memorial Hospital. The recruitment criteria for the male college students were (1) non-smokers, (2) no medication or vitamin use, and (3) no cardiopulmonary or hematological diseases. A total of 100 subjects were recruited and all provided informed consent. The subjects were instructed to refrain from exercise for $\geq$24 h before the exercise test. The subjects arrived at the testing center at 10:00 AM to eliminate any possible diurnal effect. All of the subjects completed the GXT once and 20 repeated the GXT after 48 h to confirm the test's reliability.

2.2. Graded exercise test (GXT)

Each subject underwent a GXT to determine VT_1 and VT_2. The GXT was measured using a bicycle ergometer (Corvial 400, Lode). After 2 min of unloaded pedaling, the loading was increased by 30 W every 3 min until exhaustion (Wang et al., 2010; Mao et al., 2011). Heart rate (HR), blood pressure (BP), minute ventilation (V_E), oxygen consumption (VO_2), and CO_2 production (VCO_2) were collected using a biomedical system (Powerlab, USA). Arterial O_2 saturation was monitored via finger pulse oximetry (model 9500, Nonin Onyx). The VO_{2max} criteria were (1) the level of VO_2 increased < 2 $ml \cdot kg^{-1} \cdot min^{-1}$ over $\geq$ 2 min, (2) HR exceeded the predicted 90% maximal heart rate (HR_{max}), and (3) the respiratory exchange ratio (RER) exceeded 1.2 (Wang et al., 2010; Mao et al., 2011).

2.3. Determination of VT_1 and VT_2

VT_1 was determined by an increase in V_E/VO_2 with no increase in V_E/VCO_2 and departure from the linearity of ventilation (V_E), whereas VT_2 corresponded to an increase in both V_E/VO_2 and V_E/VCO_2 (Beaver et al., 1986). All of the VT_1 and VT_2 assessments were confirmed by visually inspecting graphs of the time plotted against each relevant respiratory variable. VT_1 and VT_2 were independently determined by two experienced exercise physiologists. When the results diverged, VT_1 and VT_2 were judged by a third exercise physiologist.

2.4. NIRS skeletal muscle hemodynamics

Local skeletal muscle hemodynamics profiles were simultaneously monitored using an NIRS system (Oxymon, Artinis, Netherlands). The transmitting and receiving optode was placed on the left vastus lateralis (VL) muscle (12 cm above the proximal border of the patella and 3–5 cm lateral to the midline of the thigh) (Wang et al., 2010). The distance between the optodes was adjusted to ensure proper placement (range: 2.5–3.5 cm) and good signal strength (10–30%) and the sampling frequency was set at 10 Hz. O_2Hb and HHb were presumed to be reliable estimators of changes in tissue oxygenation or deoxygenation status representing regional O_2 delivery and utilization. Total hemoglobin (tHb) reflected changes in the tissue blood volume (Boushel et al., 2001; DeLorey et al., 2003).

2.5. Determination of VT_{1NIRS} and VT_{2NIRS}

The following procedures were used to determine tissue NIRS reflecting VT_1 and VT_2: (1) 10 Hz O_2Hb and HHb data were plotted from the beginning to the end of GXT; (2) the trend lines were drawn and adjusted using a polynomial regression model with Microsoft Excel; (3) VT_{1NIRS} was determined by the peak of O_2Hb polynomial function (the maximal value of O_2Hb); and (4) VT_{2NIRS} was determined by the peak of HHb polynomial function (the maximal value of HHb) (Fig. 1).

Polynomial regression function $\mathbf{Y = aX^3 + bX^2 + cX^1 + d}$

2.6. Statistical analysis

All of the cardiopulmonary data collected during the GXT were calculated at an average of 60 s. If there was an obvious deviation in the data, such as extreme values due to the subject sneezing or coughing, then that data were deleted. If the average exceeded 4 standard deviations, then the value was listed as an extreme value and deleted. The data were analyzed by SPSS for Windows 23.0.

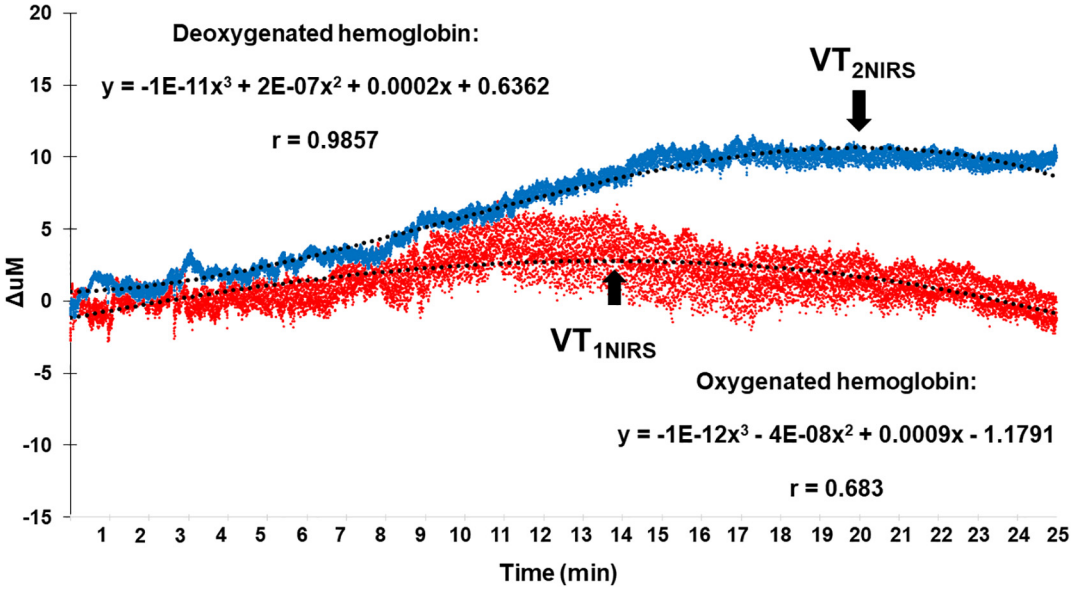

Fig. 1. NIRS signals changes during the GXT in one subject. Red dots: oxygenated hemoglobin. Blue dots: deoxygenated hemoglobin.

The independent *t*-test was used to compare VO_2 at VT_{1NIRS} and VT_{2NIRS} between the first (T_1) and second (T_2) tests for reliability. The paired *t*-test was used to compare VO_2 between VT_{1VET} vs VT_{1NIRS} and VT_{2VET} vs VT_{2NIRS}. Pearson's correlation was used to evaluate the association of VO_2 of VT_{1VET} vs VT_{1NIRS} and VT_{2VET} vs VT_{2NIRS}. A Bland-Altman analysis (Bland and Altman, 1986) was used to assess similarities between the ventilatory and NIRS methods. Statistical significance was $\alpha = 0.05$.

3. Results

The subjects' basic anthropometric and cardiopulmonary characteristics are presented in Table 1. To confirm the reliability of VT_{1NIRS} and VT_{2NIRS} during the GXT between T_1 and T_2, the correlations and similarities were assessed. There were significant positive correlations in VT_{1NIRS} and VT_{2NIRS} between T_1 and T_2. The Bland-Altman analysis demonstrated that the mean differences between VT_{1NIRS} and VT_{2NIRS} were all within a 95% (±1.96 SD) confidence interval (Fig. 2).

There were significant positive correlations between the VO_2 of VT_{1VET} vs VT_{1NIRS} and VT_{2VET} vs VT_{2NIRS}, validating VT_1 and VT_2 measured by the ventilatory and NIRS methods. The Bland-Altman analysis showed that the mean differences in VT_{1VET} vs VT_{1NIRS} and VT_{2VET} vs VT_{2NIRS} were consistent (Fig. 3). The comparisons of VO_2 between VT_{1VET} vs VT_{1NIRS} and VT_{2VET} vs VT_{2NIRS} demonstrated that there were no significant differences between the ventilatory and NIRS methods (Table 2).

4. Discussion

This study's main findings are: (1) skeletal muscle oxygenated and deoxygenated status detected via NIRS are reliable and valid for determining VT_1 and VT_2 using a mathematical polynomial regression model, (2) VT_{1NIRS} reflected the limitation of muscle oxygenation during the GXT, and (3) VT_{2NIRS} reflected the limitation of muscle deoxygenation during the GXT.

NIRS is a valid method that can be used to determine VT during exercise as previously confirmed in many populations, including normal subjects, children (Moalla et al., 2005), patients (Hamaoka et al., 2007), and athletes (van der Zwaard et al., 2016). NIRS was also confirmed as a non-invasive method of determining exercise intensity. Many studies demonstrated medium to high correlations between NIRS and traditional methods. The NIRS index demonstrated a decrease in oxyhemoglobin below baseline, a first sharp decrease in the oxygenation index, and first and second inflection early in the oxyhemoglobin slope (Bhambhani et al., 1997; Miura et al., 1998; Grassi et al., 1999; Terakado et al., 1999; Wang et al., 2006; Soller et al., 2008).

Most studies determined the NIRS index via intuitive or simple linear regression. Recent studies used more complex methods of determining the NIRS index, including tissue oxygen saturation using the Dmax method (Karatzanos et al., 2010), non-linear changes in cerebral oxygenation or deoxygenation (Racinais et al., 2014), and ΔO_2HbMb-HHbMb by double linear regression (van der Zwaard et al., 2016). The present study used NIRS origin muscle O_2Hb and HHb data to determine VT and RCP using a polynomial regression model. VT_{NIRS} and RCP_{NIRS} in this study were respectively 59.4% and 86.1%VO_{2max}. Moalla et al. (2005) investigated VT_{NIRS} using respiratory muscle oxygenation data during an incremental exercise cycling test. VT_{NIRS} was 73.8 ± 6.9% and $VT_{V\text{-}slope}$ was 69.7 ± 6.8% of VO_{2max} (Moalla et al., 2005). Karatzanos et al. (2010) studied gastrocnemius muscle oxygen saturation during an incremental treadmill test. VT was 86.2 ± 6.9%

Table 1
The subjects' basic anthropometric and cardiopulmonary characteristics.

Anthropometric		
Age (years)	22 ± 3	
Weight (kg)	67.1 ± 10.5	
Height (cm)	173.1 ± 6.0	
BMI (kg m^{-2})	22.3 ± 0.3	
Cardiopulmonary	Baseline	Peak exercise
Workload (W)	0 ± 0	210 ± 5
HR (beats min^{-1})	77 ± 3	193 ± 2
V_E (L min^{-1})	6.8 ± 0.5	127.5 ± 4.6
VO_2 (mL kg^{-1} min^{-1})	3.7 ± 0.3	54.5 ± 1.3
RER	0.85 ± 0.01	1.18 ± 0.02
SpO_2 (%)	96 ± 1	94 ± 1

Data are shown as mean ± SD. BMI: body mass index; HR: heart rate; V_E: minute ventilation; VO_2: oxygen uptake; RER: respiratory exchange rate; SPO_2: saturation of peripheral oxygen.

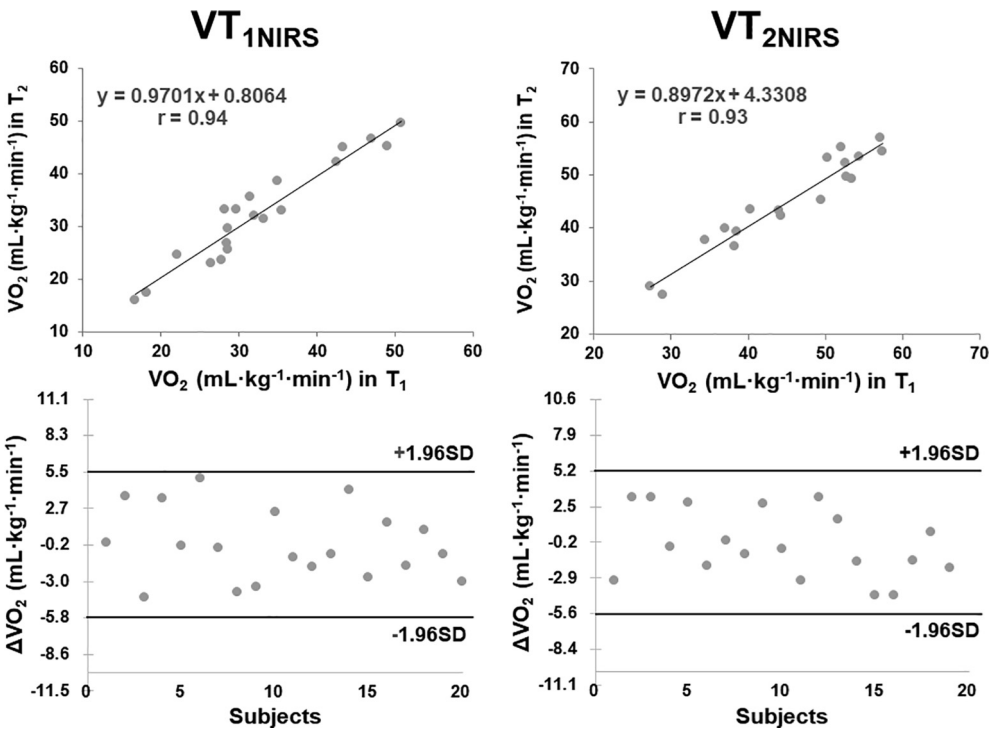

Fig. 2. Reliability analysis between the first (T_1) and second (T_2) tests (n = 20).

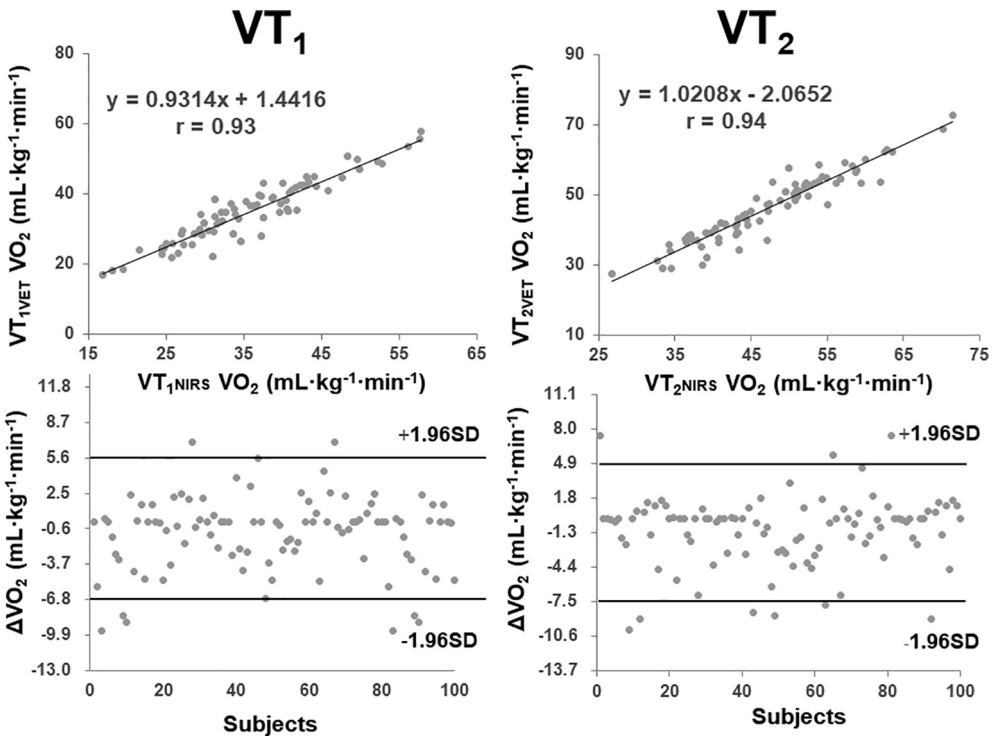

Fig. 3. Validity analysis of the ventilatory and NIRS methods (n = 100).

and NT (NIRS threshold) was 88.7 ± 7.3% VO_{2max} (Karatzanos et al., 2010). NT was determined using a linear model. Racinais et al. (2014) detected VL muscle oxygenation during an incremental cycling test. Cerebral O_2Hb was 56 ± 8% and HHb was 56 ± 13% at VT1. Cerebral O_2Hb was 86 ± 8% and HHb was 87 ± 8% at VT_2. Muscle oxygenation only related to VT2 of O_2Hb was 78 ± 9% and HHb was 80 ± 8% (Racinais et al., 2014). van der Zwaard et al. (2016) used maximal incremental cycling to detect oxygenation breakpoints via double linear regression (the least combined residual sum of squares). ΔO_2HbMb-HHbMb was 65 ± 11 $\%PO_{peak}$ in male cyclists, 62 ± 12% $\%PO_{peak}$ in female cyclists, 58 ± 12 % PO_{peak} in endurance trained males, and 62 ± 10 $\%PO_{peak}$ in

Table 2
Comparison of the cardiopulmonary parameters using the ventilatory and NIRS methods.

Parameters	VT_{1VET}	VT_{1NIRS}	VT_{2VET}	VT_{2NIRS}
Workload (W)	129 ± 3	122 ± 4	167 ± 3	159 ± 3
HR (beats min^{-1})	146 ± 1	144 ± 2	170 ± 1	165 ± 2
V_E (L min^{-1})	60.9 ± 2.1	59.5 ± 2.2	93.0 ± 2.4	87.2 ± 2.2
VO_2 (mL kg^{-1} min^{-1})	35.8 ± 1.0	35.0 ± 1.0	47.4 ± 1.0	46.2 ± 1.1
RER	1.01 ± 0.01	1.00 ± 0.01	1.09 ± 0.01	1.07 ± 0.01
SpO_2 (%)	96.1 ± 0.1	96.1 ± 0.1	96.1 ± 0.1	95.9 ± 0.2

Data are shown as mean ± SD. $^*p < .05$ calculated using the paired *t*-test to compare the ventilatory and NIRS methods. VT_{1VET}: first ventilatory threshold determined by ventilatory methods; VT_{2VET}: second ventilatory threshold determined by ventilatory methods; VT_{1NIRS}: first ventilatory threshold determined by NIRS; VT_{2NIRS}: second ventilatory threshold determined by NIRS; HR: heart rate; VO_2: oxygen uptake; V_E: minute ventilation; RER: respiratory exchange rate; S_PO_2: saturation of peripheral oxygen.

recreationally trained males. ΔO_2HbMb-HHbMb was not significantly different from VT_1 but considerably lower than VT_2 (van der Zwaard et al., 2016).

Our results were very similar to those using a non-linear regression model. This study also confirmed that VT_{1VET} and VT_{1NIRS} had a good correlation and VT_{NIR} (59.4%) occurred earlier than VT (61.3%). As the exercise intensity increased, the oxygen deficit increased accompanied by anaerobic metabolism. Lactic acid in the blood began to accumulate and bicarbonate ions inside the muscle cells increased. Increasing intracellular carbon dioxide also led to an increase in CO_2 in the blood, which was reflected by changing ventilation. The initiation of anaerobic metabolism detected by ventilation was defined as VT (Wasserman et al., 1981; Beaver et al., 1986). Our results showed that decreased muscle oxygenation (O_2Hb) was a good predictor of VT_1. Muscle O_2Hb and HHb changed according to the oxyhemoglobin dissociation curve. Lactic acid, carbon dioxide, and hydrogen ions affected the oxyhemoglobin dissociation curve and increased the dissociation of oxygen from hemoglobin. A right shift of the Bohr effect indicated an decrease in O_2Hb and a decrease in HHb in the muscle. Although VO_2 of VT_{1VET} and VT_{1NIRS} almost coincided, VT_{1NIRS} tended to occur earlier than VT_{1VET}. Our findings confirmed that VT_{1NIRS} determined using raw data with a polynomial regression is a good predictor of VT_{1VET}.

This study also confirmed that VT_{2VET} and VT_{2NIRS} have good correlation. VT_{2NIRS} (86.1%) occurred earlier than VT_{2VET} (88.6%). The definition of VT_2 was the starting point of respiratory compensation in response to exercise-induced metabolic acidosis. Beyond VT_2, the increase in hydrogen ions enhanced a shift to the right of the oxyhemoglobin dissociation curve and further increased oxygen dissociation. In this study, VT_{2NIRS} was determined by the peak of HHb function, which may have been caused by the dissociation of oxygen from myoglobin. VT_{2NIRS} also formed due to attenuated O_2Hb decrease. The decrease in O_2Hb might have increased oxygen dissociation or decreased blood flow. Our non-published data indicated that cardiac output declined during the highest intensity exercise, which might explain why the muscle blood flow also decreased as shown in the present results.

5. Conclusions

In conclusion, NIRS muscle oxygenation and deoxygenation are reliable and valid methods of determining VT_1 and VT_2 using a mathematical polynomial function model. VT_{1NIRS} reflected the limitation of muscle O_2 delivery (O_2Hb) and VT_{2NIRS} reflected the limitation of muscle O_2 utilization (HHb) during incremental cycling exercise.

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