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Research article

Differences between the *vastus lateralis* and *gastrocnemius lateralis* in the assessment ability of breakpoints of muscle oxygenation for aerobic capacity indices during an incremental cycling exercise

Bangde Wang [1,2], Guodong Xu [3,4], Qingping Tian [1,2], Jinyan Sun [1,2], Bailei Sun [1,2], Lei Zhang [1,2], Qingming Luo [1,2] and Hui Gong [1,2]✉

[1] Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, [2] Key Laboratory of Biomedical Phototonics of Ministry of Education, Huazhong University of Science and Technology, Wuhan, China, [3] School of Physical Education, Jianghan University, Wuhan, P.R.China, [4] College of Health Science, Wuhan Institute of Physical Education, Wuhan, China

Abstract

In recent years, breakpoints (Bp) of muscle oxygenation have been measured in local muscles using near infrared spectroscopy (NIRS) to assess (predict) systemic aerobic capacity indices [lactate threshold (LT), gas exchange threshold (GET) and maximal oxygen uptake (VO_{2peak})]. We investigated muscular differences in the assessment (predictive) ability of the Bp of muscle oxygenation for aerobic capacity indices during incremental cycling exercise on the aerobic capacity indices. Thirty-one active college students were recruited for an incremental cycling exercise test, during which NIRS muscle oxygenation in the *vastus lateralis* (VL) and *gastrocnemius lateralis* (GL), blood lactate concentration and cardiopulmonary variables were measured simultaneously in a multi-modality approach. A linear regression model was used to analyse the relationship between the Bp of the muscle oxygenation index (OI) and the systemic aerobic capacity indices. The Bp of the muscle OI in both the VL (BpVL) and GL (BpGL) were significantly correlated with the aerobic capacity indices. Additionally, the BpVL had a better goodness-of-fit [higher coefficient of determination (R^2, $p < 0.001$) and lower root mean squared error (RMSE, $p < 0.03$)] in the linear regressions and occurred earlier than the BpGL. In conclusion, both the BpVL and the BpGL could be measured by NIRS to assess the systemic aerobic capacity indices; however, there were muscular differences in the assessment ability of the Bp of muscle oxygenation.

Key words: Maximal incremental exercise test, aerobic capacity index, assessment ability, near infrared spectroscopy, linear regression.

Introduction

Lactate threshold (LT), gas exchange threshold (GET) and maximal oxygen uptake (VO_{2peak}) are widely accepted indicators of human aerobic exercise capacity (Bassett and Howley, 2000; Brooks, 2000; Jones and Carter, 2000). To obtain these aerobic capacity indices, the incremental exercise test (IET) (Bentley et al., 2007), accompanied by the detection of blood lactate concentration and cardiorespiratory parameters, is generally adopted (Bassett and Howley, 2000; Beaver et al., 1986; Wasserman, 1987; Wasserman et al., 1973). However, the techniques used to measure those systemic physiological parameters were either invasive (blood sample collection) or uncomfortable (involving breathing masks during cardiopulmonary function test) (Macfarlane, 2001). These disadvantages have limited the use of related techniques during the detection of aerobic exercise capacity indices.

It has frequently been reported that exercise physiologists could non-invasively evaluate relative changes in the balance between oxygen delivery and utilisation at the level of the small blood vessels—the arterioles, capillaries, and venules—by near infrared spectroscopy (NIRS) (Bhambhani, 2004; Ferrari et al., 2004). Due to its non-invasive, dynamic, and local measurement capabilities, NIRS has been broadly used to directly evaluate trends in local muscle oxygenation and blood volume during dynamic exercise (Hamaoka et al., 2007; Ozyener, 2002). Previous research has demonstrated that the breakpoints (Bp) of NIRS muscle oxygenation changes can be determined using bilinear regression and reflect the breaking up of the balance between muscle oxygen supply and consumption (Grassi et al., 1999; Wang et al., 2012). In addition, the Bp of muscle oxygenation has been found to be highly correlated with classic indicators of aerobic exercise capacity (LT, GET and VO_{2peak}) (Bhambhani, 2004; Bhambhani et al., 1997; Belardinelli et al., 1995; Grassi et al., 1999; Wang et al., 2012), which indicates that NIRS could be used to assess (predict) human aerobic exercise capacity indices non-invasively (without blood samples or wearing face masks). However, the NIRS signals have to be measured from different active muscles involved during incremental exercise, e.g., the *vastus lateralis* (VL) (Bhambhani et al., 1997; Belardinelli et al., 1995; Grassi et al., 1999; Wang et al., 2012), *gastrocnemius* (Karatzanos et al., 2010), *serratus anterior* (Legrand et al., 2007; Moalla et al., 2005), and other muscles (Rao et al., 2009). The choice of the probed muscles might influence the measurement results of muscle oxygenation changes and further influence the outcome of NIRS Bp. However, it is still unknown whether the selection of probed muscles influences the assessment (prediction) of indicators of aerobic exercise capacity by using NIRS Bp. Further study on this issue could result in guidelines for the selection of probed muscles for further use of NIRS as an alternate non-invasive method for detecting indices of aerobic exercise capacity (e.g. LT, GET and VO_{2peak}) in sports science.

To study the differences among muscles in their assessment (predictive) ability (reflected by the goodness-of-fit of the linear regression between NIRS Bp and indi-

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cators of aerobic exercise capacity, see '*Statistical analysis*') for aerobic capacity indices using muscle oxygenation Bp, active college students were recruited to participate in a maximal cycling IET. This modality was chosen because cycling is a popular recreational and competitive sport (Faria et al., 2005). During the IET, blood lactate concentration, cardiorespiratory parameters and NIRS muscle oxygenation of the thigh (VL) and calf [*gastrocnemius lateralis* (GL)] muscles were measured simultaneously. We hypothesised that: (a) there are differences between the two muscles in the Bp of muscle oxygenation between the two involved muscles; and (b) there are significant differences between different active muscles in their ability to assess aerobic capacity indices during cycling using the Bp of muscle oxygenation.

Methods

Participants

Thirty-one active college students (12 males and 19 females) were recruited from the Wuhan Institute of Physical Education. They participated in running, swimming or cycling exercise for more than 30 minutes at least 5 times a week. The mean (SE) age, height and weight for all participants were 19.7 (0.5) years, 1.76 (0.11) m and 72.7 (2.0) kg, respectively. Additionally, the subcutaneous adipose thickness at the sites of the NIRS probes was measured with a caliper and was 7.2 (2.6) mm and 6.1 (2.7) mm for VL and GL, respectively. All participants were free from metabolic and cardiorespiratory disorders. Before the IET, written informed consent was obtained from each participant as directed by the local ethics committee, according to the standards established in the Declaration of Helsinki. Additionally, all participants were allowed to withdraw from the study without any restrictions during the tests.

Exercise protocol

Before the experiment, the seat height of an electronically braked bicycle ergometer (Lode Examiner, Lode VL, Groningen, Netherlands) was adjusted for each participant to achieve a slight bend in the knee when the right foot was at the bottom of the pedal movement. During the IET, after a 3-min rest seated on the ergometer, the participants performed an incremental cycling exercise at a pedalling rate of 60 rpm. The incremental exercise began at an initial workload (100 W for male, 40 W for female, considering the fitness levels of recruited participants and avoiding an overly long duration of IET), followed by increments of 30 W every 3 min (Bentley et al., 2007; Roffey et al., 2007) until volitional fatigue or two of the following criteria (Bhambhani et al., 1998; Zhang et al., 2010) were attained: (a) heart rate (HR) ≥ age-predicted maximal HR, which was calculated as 220 minus age (in years); (b) no further increase in oxygen uptake (VO_2) occurred with increasing workload (increase of less than 100 $mL \cdot min^{-1}$); or (c) respiratory exchange ratio (RER) ≥ 1.10. Muscle oxygenation monitoring, blood lactate testing and cardiorespiratory measurements were performed simultaneously during both rest and incremental exercise.

Cardiorespiratory measurements

Respiratory gas exchange variables, such as minute ventilation (V_E), VO_2, carbon dioxide output (VCO_2) and RER were recorded using a metabolic system (MAX II, Physio-Dyne Instrument Corp., New York, USA). The oxygen and carbon dioxide analysers in the system were calibrated using commercially available precision gases (100% nitrogen for the low calibration process; 21% oxygen, 5% carbon dioxide, and the balance as nitrogen for the high calibration process). The volume of the mass flow sensor was calibrated using a 3-L syringe as recommended by the manufacturer. Heart rate (HR) was recorded using a heart rate detector that is a part of the MAX II, and the signals were received in the form of output pulses of a Polar transmitter and receiver. HR and respiratory gas exchange variables were recorded continuously and averaged every 10 s.

The GET was identified by the V-slope method (Beaver et al., 1986; Sekir et al., 2002; Yasuda et al., 2006), which was based on the determination of the nonlinear point of increase in the slope of VCO_2 versus VO_2 during incremental exercise.

Blood lactate concentration test

Blood lactate concentration ($[La]_b$) was measured using a portable lactate test meter (Lactate Pro^{TM}, LT-1710, Arkray, Shiga, Japan) (Mc Naughton et al., 2002). During the last 30 s of each 3-min, 5µl of blood was sampled from fingertips for $[La]_b$ analysis. $[La]_b$ was interpolated every 10 s to be compared with cardiopulmonary variables. The LT was detected using the log-log method (Davis et al., 2007).

NIRS muscle oxygenation monitoring

The theory and application of NIRS for measuring muscle oxygenation have been extensively described elsewhere (Ferrari et al., 2004; Hamaoka et al., 2007). In this study, a homemade two-channel continuous wave (CW) NIRS muscle oxygenation device (Wang et al., 2012; Zhang et al., 2009; 2010) was used to measure the muscle oxygenation in the right VL and GL simultaneously during the tests. Each probe of the CW-NIRS device consisted of one light source and one detector. The light source integrated 3 kinds of light-emitting diodes (LED) with wavelengths of 730, 805 and 850 nm. The light at a 730 nm wavelength is primarily absorbed by deoxygenated haemoglobin (HHb) chromophores when it penetrates the tissue, while at an 850 nm wavelength the main absorption chromophores are in oxygenated haemoglobin (O_2Hb). The 805 nm wavelength is the isosbestic point of the absorption coefficients of O_2Hb and HHb. The light intensity changes at 805 nm could be used to calculate the concentration changes in total haemoglobin (tHb), which is considered to be an indicator of changes in blood volume. The light intensity before and after absorption and scattering by the tissue was recorded by the CW-NIRS muscle oxygenation device, and the absorbance could then be calculated. Calculated from some functions of absorbance at adopted wavelengths (Lin et al., 2002; Wang et al., 2012), the relative concentration changes of HHb, O_2Hb, and tHb can be obtained according to the

Beer–Lambert law (Hamaoka et al., 2007; Zhang et al., 2010) and were termed Δ[O_2Hb], Δ[HHb], and Δ[tHb]. The difference between the relative concentration changes in O_2Hb and HHb (Δ[O_2Hb-HHb]) was taken as the muscle oxygenation index (OI) (Hamaoka et al., 2007; Legrand et al., 2007).

The probes of the CW-NIRS muscle oxygenation device were secured directly over the motor point of the right VL (along the vertical axis of the thigh, approximately 10-12 cm above the knee joint) and the right GL (18-20 cm below the knee, parallel to the major axis of the GL) (Bhambhani et al., 1997; Grassi et al., 1999; Hiroyuki et al., 2002). Each probe was wrapped by an elastic bandage around the lower limb without occluding the blood flow (Bhambhani et al., 1997; Grassi et al., 1999). The distance between the light source and the detector in the probe was set to be 35 mm and the depth of light penetration would be more than half of that (Hamaoka et al., 2007). The NIRS signals were collected at a sampling frequency of 2.9 Hz. All data were expressed in arbitrary units (AU) with the resting values as zero (Ferrari et al., 2004; Zhang et al., 2010).

The Bp of OI in the VL (BpVL) and GL (BpGL) at which a significant change in the OI slope occurred was determined by iteratively fitting different combinations of two regression lines to contiguous experimental points obtained during the incremental exercise and by determining which combination yielded the lowest sum of squared residuals (Figure 1) (Grassi et al., 1999).

Statistical analysis

All data were expressed as the mean (SE) unless indicated otherwise. The correlation relationships were evaluated using Pearson's product-moment correlation (Atkinson and Nevill, 1998). Paired-samples *t* tests were used to analyse the differences among local muscle thresholds (BpVL and BpGL), systemic thresholds (LT and GET) and peak values at exhaustion. To study the assessment (predictive) ability of muscle oxygenation breakpoints, the BpVL and the BpGL were used separately as explanatory variables to assess (predict) the explained variables (LT, GET or VO_{2peak}) one by one using a linear regression model (y = b*x + c) with the least squares method. The degree-of-freedom adjusted coefficient of determination (R^2_a), the proportion of the variation in the explained variable that can be explained by the explanatory variable) and the root mean squared error (RMSE, the square root of the average squared distance of a data point from the fitted line) of the linear regression were calculated to evaluate the assessment ability (Korn and Simon, 1991; Srivastava et al., 1995). Higher R^2_a and lower RMSE would indicate a higher ability of the Bp of muscle oxygenation to assess indices of aerobic exercise capacity (Agresti and Franklin, 2009; Bender, 2009). To compare the assessment ability between BpVL and BpGL, a paired-samples *t* test was used to analyse the difference in R^2_a and RMSE between the two breakpoints. Statistical significance was accepted at $p < 0.05$ unless otherwise specified. All statistical analyses were performed using the SPSS (Statistical Package for the Social Sciences) computer programs.

Results

Peak values of physiological variables and work rate

All participants completed the required tests and met the criteria for maximal exercise mentioned in '*Exercise protocol*'. Due to technical problems, the $[La]_b$ analysis failed in 3 male and 4 female participants. Peak values obtained during the exhausting workload [WR_{peak}, 210 (6) W] for ventilatory and metabolic variables are listed in Table 1. VO_{2peak} was 3.9 (0.2) L·min^{-1} or 53.4 (2.2)

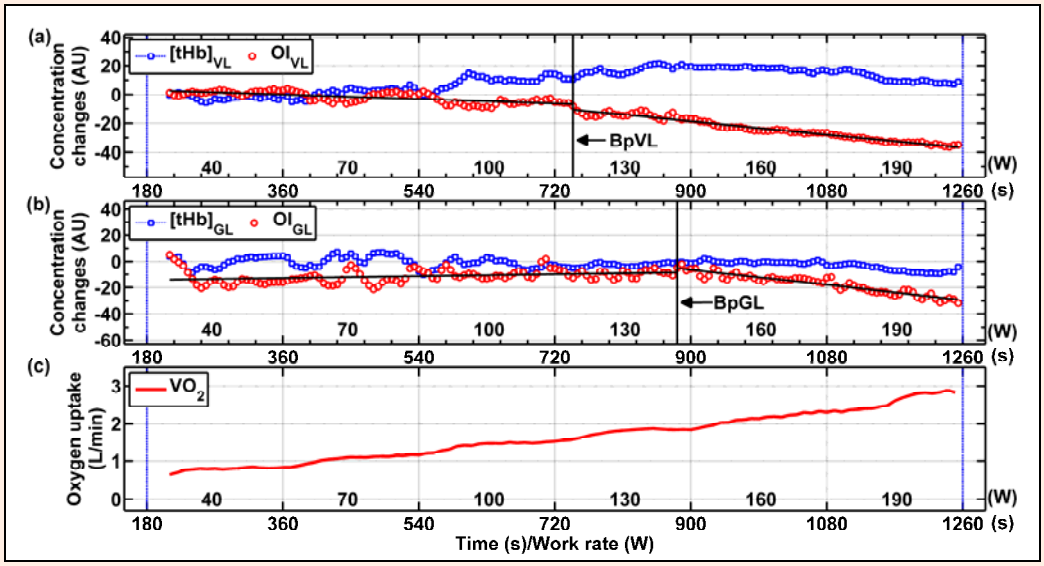

Figure 1. The trends of NIRS variables and oxygen uptake as a function of time/work rate. A typical result from a female participant is presented. Δ[tHb], the change in total haemoglobin concentration; OI, the oxygenation index; VL, *vastus lateralis*; GL, *gastrocnemius lateralis*; AU, arbitrary unit; VO_2, oxygen uptake. The vertical blue dotted lines indicate the start and the end of the incremental cycling exercise; the vertical black solid lines in subgraph (a) and (b) indicate the breakpoints (Bp) of OI in the VL (BpVL) and GL (BpGL); significant changes in slope of OI decrease during exercise (BpVL and BpGL) were identified by calculating combinations of linear regressions [black solid lines shown in subgraph (a) and (b)] that yielded the lowest sum of squared residuals.

$mL \cdot min^{-1} \cdot kg^{-1}$. The $[La]_b$ at exercise exhaustion ($[La]_{bpeak}$) for all participants was 8.8 (0.5) mM. Additionally, the WR_{peak} was significantly correlated with VO_{2peak} ($L \cdot min^{-1}$) ($r = 0.884$, $p < 0.001$).

Time, work rate and physiological responses at metabolic thresholds

The statistical results of time, physiological variables and work rate (WR) at the four metabolic thresholds (BpVL, BpGL, LT, and GET) and exercise exhaustion (Peak) for all participants are listed in Table 1. Significant correlations among the four thresholds were found when they were expressed in WR (W) ($r > 0.824$, $p < 0.001$) and VO_2 ($L \cdot min^{-1}$) ($r > 0.839$, $p < 0.001$). The WR and VO_2 ($L \cdot min^{-1}$) at the four thresholds were highly correlated to WR_{peak} ($r > 0.854$, $p < 0.001$) and VO_{2peak} ($r > 0.846$, $p < 0.001$). Additionally, all physiological thresholds were different from peak values ($p < 0.001$).

Muscular differences in muscle oxygenation breakpoints

The % VO_{2peak} for all participants at the BpVL [57.7 (1.4) %] was significantly lower than that at the BpGL [65.7 (1.7) %] ($p < 0.001$). There was also a difference between BpVL and BpGL for time, work rate and other physiological variables ($p < 0.01$, Table 1). The coefficients (b and c), R^2_a, and RMSE of linear regression are listed in Table 2. R^2_a was higher ($p < 0.001$) and RMSE was lower ($p = 0.03$) when the BpVL was used as a regressor.

Discussion

In this study, a multi-modality approach was adopted to simultaneously monitor the local and systemic physiological changes from a single maximal cycling IET in each participant. This design is important because the status of the same participant might differ during different tests. Therefore, our study design allowed better elucidation of the muscular differences in muscle oxygenation, in addition to the relationships among the local and the systemic physiological changes that occur during maximal exercise. Although the local metabolic thresholds of both muscles (BpVL and BpGL) were correlated to the indicators of aerobic exercise capacity, the BpVL occurred earlier and had a higher R^2_a and lower RMSE than the BpGL during the linear regression.

Relationships among the muscle oxygenation breakpoints and the aerobic capacity indices

The breakpoints of the muscle oxygenation index were identified at the work intensity at which a change in the slope of OI ($\Delta[O_2Hb\text{-}HHb]$) occurred. Similar to a previous study (Grassi et al., 1999), these breakpoints also corresponded to the work intensity at which $\Delta[O_2Hb]$ started to decrease (*data not shown*). It should be noted that OI can be treated as a reliable oxygenation index only if $\Delta[tHb]$ is constant. In the present study, an accelerated OI decrease was found in the presence of a nondecreasing $\Delta[tHb]$ (Figure 1), thereby indicating true deoxygenation. In brief, both the BpVL and the BpGL indicated the imbalance between oxygen delivery and demand.

The relationships among BpVL, BpGL, LT, and GET are still unclear because the four thresholds have not been determined simultaneously in previous studies. In this study, a multi-modality approach was adopted, and significant correlations were found between the BpVL and aerobic capacity indices (LT, GET, and VO_{2peak}), which is in agreement with previous reports (Bhambhani et al., 1997; Grassi et al., 1999; Wang et al., 2012). Additionally, there were significant correlations between the BpGL and the two systemic thresholds, which was consistent with a recent report that differed in the exercise modality used (treadmill exercise) (Karatzanos et al., 2010). Similar to BpVL, the BpGL was significantly correlated to WR_{peak} and VO_{2peak}, indicating that the GL could also be used for non-invasively detecting local anaerobic thresholds during cycling IET. Additionally, a significant correlation was found between the BpVL and the BpGL. Although the GL is one of the main muscles during cycling exercise, both the existence of muscle oxygenation breakpoints in the GL and the relationship between the BpGL and aerobic capacity indices have rarely been reported. Previous studies monitored the surface electromyography (sEMG) signal changes in both the VL and the

Table 1. The time, work rate and physiological responses corresponding to four physiological thresholds and the peak values during incremental cycling exercises. Data are expressed as the mean (SE).

Variables	BpVL	BpGL	LT	GET	Peak
n	31	31	24	31	31
Time (s)	797 (25)*†‡	895 (23)	899 (27)‡	933 (26)	1256 (32)
WR (W)	137 (6)*†‡	153 (6)	154 (7)‡	160 (6)	213 (7)
V_E ($mL \cdot min^{-1}$)	60.2 (3.1)*†‡	70.8 (3.8)	68.8 (4.4)	72.0 (3.7)	134.3 (7.5)
VO_2 ($L \cdot min^{-1}$)	2.2 (0.1)*†‡	2.5 (0.1)	2.5 (0.2)‡	2.6 (0.1)	3.9 (0.2)
VO_2 ($mL \cdot min^{-1} \cdot kg^{-1}$)	30.6 (1.3)*†‡	34.7 (1.5)	34.6 (1.7)‡	36.5 (1.5)	53.4 (2.2)
VCO_2 ($L \cdot min^{-1}$)	2.1 (0.1)*†‡	2.5 (0.1)	2.5 (0.2)	2.6 (0.1)	4.3 (0.2)
RER	0.96 (0.02)*†‡	0.99 (0.02)	0.98 (0.02)	0.98 (0.02)	1.13 (0.02)
HR (bpm)	144 (2)*†‡	154 (3)	152 (2)‡	157 (2)	184 (2)
[La]b (mM)	3.7 (0.3)*†‡	4.2 (0.3)	3.6 (0.2)‡	4.5 (0.3)	8.8 (0. 5)
%VO_{2peak} (%)	57.7 (1.4)*‡	65.7 (1.7)	64.5 (1.6)‡	68.7 (1.3)	--

n, sample size; WR, work rate; V_E, ventilation; VO_2, oxygen uptake; VCO_2, carbon dioxide output; RER, respiratory exchange ratio; HR, heart rate; $[La]_b$, blood lactate concentration; % VO_{2peak}, percentage of VO_{2peak}; BpVL and BpGL, the breakpoints (Bp) of the muscle oxygenation index in the *vastus lateralis* (BpVL) and *gastrocnemius lateralis* (BpGL); LT, lactate threshold; GET, gas exchange threshold; Peak, peak values during the incremental exercise. *, significantly different from BpGL ($p < 0.05$); †, significantly different from LT ($P < 0.05$); ‡, significantly different from GET ($p < 0.05$). All thresholds (BpVL, BpGL, LT and GET) were significantly different from the Peak values ($p < 0.05$).

Table 2. The linear regression relationship between local thresholds and systemic aerobic capacity indices.

Explained variable (y)		Explanatory variable (x) = BpVL				Explanatory variable (x) = BpGL			
		b	c	R^2_a *	RMSE*	b	c	R^2_a	RMSE
LT	VO_2 ($L \cdot min^{-1}$)	1.038	.199	.800	.334	.871	.314	.691	.415
	VO_2 ($mL \cdot min^{-1} \cdot kg^{-1}$)	.942	5.622	.703	4.546	.779	7.351	.597	5.295
	WR (W)	.980	17.573	.774	16.535	.893	15.266	.665	20.161
GET	VO_2 ($L \cdot min^{-1}$)	1.093	.221	.856	.280	.909	.363	.741	.376
	VO_2 ($mL \cdot min^{-1} \cdot kg^{-1}$)	1.061	4.046	.795	3.833	.846	7.129	.660	4.937
	WR (W)	1.010	21.271	.864	12.513	.890	23.152	.689	18.964
Peak	VO_2 ($L \cdot min^{-1}$)	1.583	.362	.803	.490	1.327	.542	.706	.598
	VO_2 ($mL \cdot min^{-1} \cdot kg^{-1}$)	1.093	.221	.856	.280	.909	.363	.741	.376
	WR (W)	1.083	64.886	.773	18.348	1.030	55.415	.721	20.337

LT, lactate threshold; GET, gas exchange threshold; Peak, peak values during the incremental exercise; Linear regression ('y=b*x+c') with the least squares method was used to analyse the relationship between local thresholds (BpVL and BpGL) and systemic aerobic capacity indices (LT, GET and VO_{2peak}) when they were expressed as corresponding VO_2 and WR; R^2_a, degree-of-freedom adjusted coefficient of determination; RMSE, root mean square error (standard error). *, paired-samples *t* tests showed significant differences in R^2_a ($p < 0.001$) and RMSE ($p = 0.03$) between the explanatory variables (x) (BpVL and BpGL).

GL, and the sEMG threshold appeared in both muscles (Hug et al., 2006), which is consistent with the occurrence of the Bp of OI in the two muscles in this study. Although the occurrence rate of the sEMG threshold in GL was lower than that in VL (Hug et al., 2006), the occurrence rate of Bp of OI in both VL and GL was 100% in this study.

From a physiological perspective, the dissociation of O_2Hb in local muscles should speed up to meet the increasing ATP requirements at mild intensity exercise in the IET. However, a previous study showed that more O_2 was transferred into the active muscles by an increase in blood flow (Sorensen et al., 2000), which may explain why the OI decreased slightly but not significantly. With further increasing workload, more O_2 was extracted from O_2Hb, which could be detected by the continuously decreasing OI without a significant decrease in Δ[tHb] (Figure 1). During exercise of mild to moderate intensity, lactic acid is produced in skeletal muscles even under fully aerobic conditions (Brooks, 2000); however, most of the lactic acid could be reoxidized in the skeletal muscle and other less active muscles (Gladden, 2000). Therefore, the production and removal of lactic acid is comparable and the net lactic acid diffusing into the blood is not high, and the $[La]_b$ remains constant or only slightly increases. To meet the ATP demands during intensive workloads, more glycolytic muscle fibres must be recruited, resulting in the accumulation of lactic acid in the local exercising muscles. Lactic acid can dissociate into lactate and H^+. The increase in the concentration of H^+ accelerates the O_2Hb dissociation via the Bohr effect and consequently accelerates the decrease in OI (Stringer et al., 1994). When lactic acid production exceeds lactic acid removal in the muscles, blood lactic acid increases nonlinearly (Yoshida et al., 1982) and more blood lactic acid dissociates, inducing significant increases in $[La]_b$. The accumulated H^+ is also buffered by bicarbonate or other buffers in the systemic blood, resulting in the production of carbonic or other acids. Carbonic acid dissociates into water and CO_2. Both the excess CO_2 and the increased H^+ concentration can stimulate the central chemoreceptors and cause a nonlinear increase in ventilation (V_E), and the extra ventilation causes excretion of the extra CO_2, resulting in a nonlinear increase in the slope of VCO_2 versus VO_2 during incremental exercise. These steps may account for the correlations of the four physiological thresholds.

Muscular differences in breakpoints of muscle oxygenation changes

Considering that the VL and GL were mostly studied independently in previous NIRS studies, we tested the VL and GL simultaneously to compare the two muscles, as the VL and GL are involved as a knee extensor and a knee flexor/ankle stabiliser, respectively, during cycling. The BpVL appeared earlier than the BpGL ($p < 0.001$), indicating that the oxygen supply-consumption balance in the VL was broken earlier than that in the GL during cycling IET. Furthermore, the BpVL had higher assessment ability (indicated by the higher R^2_a and lower RMSE, Table 2) for the aerobic capacity indices than the BpGL. One reason for the differences in the Bp of OI between the VL and GL might be the differences in anatomical and histochemical characteristics. Sufficient evidence has shown that the percentage of type I (slow twitch) fibres in the VL is lower than that in the GL (Edgerton et al., 1975; Houmard et al., 1998; Staron et al., 2000). Additionally, the activity of oxidative enzyme (citrate synthase) was previously reported to be lower in the VL than that in the GL (Houmard et al., 1998). Due to the lower percentage of type I fibres and lower activity of oxidative enzymes in the VL, the fast twitch fibres would be largely recruited earlier in the VL when the workload continuously increases, resulting in more anaerobic metabolism in the VL during moderate and high intensity exercise. The earlier accumulation of acidic metabolic substances in the VL might result in more H^+ and a lower pH. Due to the Bohr effect, the accelerated dissociation of O_2Hb would occur earlier in the VL due to the earlier accumulation of acidic metabolic substances; this result is indicated in our data by the earlier breaking up of the oxygen supply-consumption balance in the VL (BpVL). Another reason for the differences in the Bp of OI between VL and GL might be different usage patterns of the muscles during cycling. The mono-articular muscles (e.g., the VL) are primarily involved in the generation of positive work, whereas the biarticular muscles (e.g., the GL) are responsible for regulating force transmission during cycling (So et al., 2005). Additionally, the VL is thought to be one of

the most active muscles during cycling (Hug et al., 2006) and seems to produce more muscle work than the GL over the crank cycle (Neptune et al., 2000). Therefore, the contribution of the VL is most likely higher than that of the GL during cycling, which might account for the earlier occurrence of the BpVL and the higher assessment ability of the BpVL for the aerobic exercise capacity indices. In summary, the differences in the BpVL and the BpGL might be mostly associated with the muscular differences in the percentage of muscle fibres and the usage patterns during cycling. However, further research with muscular biopsy and/or sEMG is needed to confirm this type of association.

The multi-modality approach is useful for providing guidelines for the selection of probe muscles during the evaluation of aerobic exercise capacity by NIRS. In the present study, the BpVL was a better assessor (predictor) of systemic aerobic exercise capacity indices when compared with BpGL during cycling IET. In other words, the VL should have a higher priority for selection when local muscles can be measured by NIRS to assess (predict) indices of systemic aerobic exercise capacity during cycling IET. Only two muscles were measured and compared in this study, therefore, more muscles recruited during pedalling should be studied in further studies. Additionally, considering the differences in muscle usage patterns during different types of exercise, using a multi-modality approach to measure more muscles should be adopted with other types of exercise to establish guidelines for specific exercises.

Conclusion

In this study, the NIRS variables in two muscles, blood lactate concentration and cardiorespiratory variables were monitored simultaneously during maximal cycling IET. The local muscle thresholds were highly correlated with the aerobic capacity indices, while the BpVL had better goodness-of-fit in linear regressions of local Bp of muscle OI with the systemic aerobic capacity indices. These correlations indicated that both the VL and GL could be used to assess the aerobic capacity indices non-invasively by NIRS, while there were differences between the muscles in their assessment abilities on the aerobic capacity indices. The multi-modality approach in this study is useful for providing guidelines for the selection of probe muscles for evaluation of indices aerobic exercise capacity by NIRS.

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Key points

- The breakpoints (Bp) of muscle oxygenation index in both *vastus lateralis* (VL) and *gastrocnemius lateralis* (GL) could be detected to indicate the breaking up of the oxygen supply-consumption balance by NIRS.
- The Bp of muscle oxygenation index in both VL (BpVL) and GL (BpGL) were significantly correlated with the systemic aerobic capacity indices.
- The BpVL owned higher assessment (predictive) ability when the Bp (BpVL and BpGL) of muscle oxygenation index was used to assess (predict) systemic aerobic capacity indices.

AUTHORS BIOGRAPHY

Bangde WANG
Employment
PhD Candidate, Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, China
Degree
BE
Research interests
Biomedical Engineering, Near Infrared Spectroscopy, Exercise Physiology.
E-mail: bdewang@gmail.com

Guodong XU
Employment
Professor, College of Health Science, Wuhan Institute of Physical Education, China.
Degree
PhD
Research interests
Sport Science, Near Infrared Spectroscopy, Exercise Physiology.

Qingping TIAN
Employment
PhD Candidate, Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, China
Degree
BE
Research interests
Near Infrared Spectroscopy, Exercise Physiology.

Jinyan SUN
Employment
PhD Candidate, Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, China
Degree
BE

Research interests
Near Infrared Spectroscopy, Brain and Cognitive Sciences.

Bailei SUN
Employment
PhD Candidate, Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, China
Degree
BE
Research interests
Near Infrared Spectroscopy, Brain and Cognitive Sciences.

Lei ZHANG
Employment
PhD candidate, Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, China
Degree
BE
Research interests
Biomedical Engineering, Near Infrared Spectroscopy, Brain and Cognitive Sciences.

Qingming LUO
Employment
Professor, Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, China
Degree
PhD
Research interests
Biomedical Photonics, Biomedical Engineering, Optical Engineering.

Hui GONG
Employment
Professor, Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, China
Degree
BE
Research interests
Biomedical Photonics; Exercise Physiology; Brain and Cognitive Sciences.
E-mail: huigong@mail.hust.edu.cn

✉ **Hui Gong**
Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, China