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Respiratory Muscle Deoxygenation and Ventilatory Threshold Assessments Using Near Infrared Spectroscopy in Children

Abstract

The aim of this study was to assess respiratory muscles deoxygenation and to determine ventilatory threshold using near infrared spectroscopy (NIRS) in children during incremental cardio-pulmonary exercise. Fourteen healthy children with a mean $\pm$ SD age of 12.8 ± 1.4 yrs performed an incremental exercise test on a cycle ergometer. NIRS was used to assess deoxygenation of the respiratory muscles. Ventilatory parameters (oxygen uptake, carbon dioxide production, and ventilation minute), power output, and tissue saturation (StO_2) were measured. Ventilatory threshold was determined by the two following methods: the V-slope method which corresponds to the breakpoint in $\dot{V}CO_2$ as a function $\dot{V}O_2$ relationship ($VT_{V-slope}$) and the NIRS method which corresponds to the point of rapid fall in StO_2 (VT_{nirs}). Dur-

ing exercise, the respiratory muscles deoxygenated as the exercise intensity increased. StO_2 decrease progressively until an abrupt decrease was observed. No significant differences were observed between cardiorespiratory variables corresponding either to $VT_{V-slope}$ or to VT_{nirs} . The two methods showed a good agreement (data were inside the 95% confidence interval). Likewise, a significant relationship was found between $VT_{V-slope}$ and VT_{nirs} for each parameter measured ($r = 0.87$ to 0.94 , $p < 0.001$). We concluded that respiratory muscles deoxygenate during incremental exercise in children and that ventilatory threshold could be determined by NIRS.

Key words

NIRS · incremental exercise test · threshold determination · rib cage muscles

Introduction

Evaluation of physiological responses and assessment of exercise tolerance have usually been focused on the measurement of peak oxygen uptake ($\dot{V}O_{2max}$) [11,30]. Nevertheless, peak performance is more dependent on the subjects' motivation than submaximal performance, especially in children [1,14]. The common measurement of submaximal intensity is the determination of ventilatory threshold (VT) [17,22]. VT is defined as "the exercise intensity at which the increase in ventilation becomes disproportional to the increase in power output during an incremental ex-

ercise test" [32]. At this point, the muscle requirement for oxygen begins to exceed that provided by the cardiovascular system. Consequently, lactate accumulates and acidosis, hyperventilation, and impaired exercise capacity are induced [17,27]. Wasserman et al. [36] explained accumulation of lactates by altered oxygen kinetics, hence tissue hypoxia. Various methods of determining VT have previously been used; ventilatory equivalents, excess CO_2 production, lactate kinetics, etc. However, the simplest and the most common method used is the V-slope method [4] which is based on the determination of the nonlinear point of

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increase in slope of carbon dioxide production ($\dot{V}CO_2$) versus oxygen uptake ($\dot{V}O_2$) during incremental exercise.

The physiological changes from VT as lactate accumulation, metabolic acidosis, impaired muscle contraction, hyperventilation, altered oxygen kinetics [27] take place in peripheral muscles, but also in respiratory muscles whose work increases by several mechanisms [2]. In fact, the decrease in oxygen transport and ATP generation to the respiratory muscles during exercise has been described as the important causative mechanism in muscle fatigue because of lactic acid accumulation [16]. Thus, respiratory muscle function depends directly upon the ability to deliver an adequate supply of oxygen and substrates to support contractile activity [20].

Near infrared spectroscopy (NIRS) is a noninvasive and continuous method that allows the monitoring of oxygenation and blood volume in muscular tissue. Application, theoretical basis, advantages, and limitations of NIRS have been widely documented [5,23,25]. NIRS has often been used to evaluate peripheral muscle, especially the vastus lateralis. To the best of our knowledge, only two studies were interested in the respiratory muscles to assess deoxygenation [24,33]. Moreover, no study in the literature has employed NIRS in the measurements of oxygenation in children.

Because respiratory muscles contribute to exercise limitation and because oxygenation depends on the balance between oxygen consumption and oxygen supply [3], we hypothesized that: firstly, respiratory muscles deoxygenate during incremental cardiopulmonary exercise; secondly, NIRS could be used as an additional tool to assess respiratory muscles and to determine VT.

Materials and Methods

Subjects

Fourteen healthy boys (age, 12.8 ± 1.4 yr; height, 155.1 ± 8.7 ; weight, 46.4 ± 7.7 kg) participated in this study. All subjects practiced recreational sports and physical activity only at school. The children and their parents signed a written informed consent in accordance with the ethical standards of the Helsinki Declaration of 1975.

Lung function testing

Measurements of pulmonary function were performed on a spirometer (Master Lab, Jaeger, Germany). The forced expiratory volume in 1 second (FEV_1), the forced vital capacity (FVC), and the total lung capacity (TLC) were determined. Results were expressed as absolute values and as percentage of predicted reference values (%pred) according to Quanjer et al. [28]. The FEV_1/FVC ratio was then calculated.

Exercise testing protocol

Each subject underwent an incremental exercise test with a constant pedalling cadence of 60 revolutions/min (rpm) on an electromagnetically braked cycle ergometer (Jaeger Ergoline, ER800, Germany). The increase in workload was individualized and was calculated according to the Wasserman norms as a function of sex, weight, and age of each child [37]. The test involved four

consecutive periods: (1) a 3-min rest period, (2) a 2-min period of warm-up against a workload corresponding to 20% of the theoretical maximal power output, (3) an 8-to 12-min exercise period with increases in the workload every minute until exhaustion, and (4) a recovery period. Standardized encouragements were given to the children to develop their maximum effort. Tests were considered maximal when at least three of the following criteria were achieved: (1) stabilization of $\dot{V}O_2$ despite a further increase in workload, (2) the respiratory exchange ratio (RER) greater than 1.1, (3) a maximal heart rate (HR) close to the predicted maximal HR; and (4) the inability to maintain the pedalling speed (60 rpm).

Pulmonary gas exchange

Pulmonary ventilation ($\dot{V}E$), $\dot{V}O_2$, and $\dot{V}CO_2$ were determined breath-by-breath by means of a respiratory gas analyzing system (MGA 1100 Medical, Marquette Electronics, USA). The subjects wore a nose clip and breathed through a mouthpiece (Hans-Rudolph). Before each test, gas analyzers were calibrated with known reference gases (12% O_2 , 5% CO_2 , and 83% N_2). The HR was monitored through the test and recorded continuously by electrocardiogram (12 SL Program, Marquette Electronics, USA).

NIRS measurements

The changes in respiratory muscle saturation (StO_2) were evaluated by NIRS (Runman, NIM, Philadelphia) consisting of a probe and a main unit. The probe, molded in elastic black silicone rubber, has a photodetector in the center and two light-emitting diodes (wavelengths of 760 and 850 nm) on either side. The distance between diodes and photodetectors was 3 cm from each other. The depth of penetration of near infrared light is approximately equal to half the distance between light source and detector, i.e., 1.5 cm [25]. NIRS measurement is based on the relative tissue transparency for light in the near infrared region and on the O_2 -dependent absorption changes of hemoglobin (Hb) and myoglobin (Mb). However, it has been reported that the major signal from NIRS comes from Hb [23]. The values obtained reflect the balance between oxygen consumption and oxygen supply [26]. Changes in muscle oxygen saturation were estimated according to the difference in the two wavelengths (760 nm–850 nm).

To record the NIRS signal, the probe was placed on the sixth intercostal space, at the anterior axillary line over the serratus anterior muscle as described by Mancini et al. [24] and Terakado et al. [33]. Concomitantly to the diaphragm, this superficial muscle serves as an accessory inspiratory muscle during ventilation to elevate ribs. A permanent marker was used on the skin to identify the location where the probe facing the muscle was placed, in order to ensure that it was invariant and was not displaced. A tape of clear plastic was placed around the probe to protect the photodetector from sweat and moisture and secured by straps and adhesive tape. In addition, an elasticised cloth was used around the chest to avoid movement of the probe. This preparation essentially prevented any probe movement relative to the skin surface. The data was displayed continuously on a monitor in real time and stored for further analysis.

After installing the subject on the ergocycle and prior to the test protocol, the NIRS was calibrated at 760 nm and 850 nm accord-

ing to the manufacturer's instructions and specifications. Calibration was repeated before each test for each subject until the NIRS baseline value was obtained. The signal measured by NIRS indicates only qualitative data. Previous investigations have often employed arterial occlusion as a physiological scale to determine the level of oxygenation [3,23,26]. However, this method cannot be applied in the case of respiratory muscles. We therefore used the method described by Belardinelli et al. [5]; for changes in StO_2 , the lowest value obtained during incremental exercise test was taken as the minimum saturation, 0%, whereas the maximal value recorded during recovery was taken as maximal saturation, 100% (maximal hyperemia); thus, StO_2 values were expressed according to this scale.

Determination of the ventilatory threshold

After completion of each incremental test, the ventilatory threshold (VT) was determined for each subject using the V-slope method ($VT_{V-slope}$) described by Beaver et al. [4]. This method involves the behaviour analysis of $\dot{V}CO_2$ as a function of $\dot{V}O_2$ during exercise. VT corresponds to the breakpoint of the linearity of the curve $\dot{V}CO_2$ versus $\dot{V}O_2$ (Fig. 1a). Likewise, VT was determined by NIRS (VT_{nirs}) as the intensity at which an accelerated fall in tissue oxygenation was observed. This intensity is represented on the graph by the point of nonlinear decrease in oxygen saturation versus $\dot{V}O_2$ (a breakpoint of the linearity of the curve also) (Fig. 1b). These two points of $VT_{V-slope}$ and VT_{nirs} were determined randomly from graph visually on the computer by two experienced investigators having no prior knowledge of any results or subject identities. In the rare cases of disagreement, a third person was used to establish a consensual determination. Similarly, in order to reflect mechanical load of respiratory muscles, a similar breakpoint was observed when we plotted StO_2 as a function of $\dot{V}E$ (Fig. 1c).

Data analysis

StO_2 and cardiorespiratory responses (HR, $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$) were examined every minute at each stage. These variables were also determined separately at VT as detected by the $VT_{V-slope}$ and VT_{nirs} methods. For reliability and validation, Bland-Altman [8] plots were used to show the agreement between the two methods ($VT_{V-slope}$ and VT_{nirs}) for each parameter by evaluating their mean differences against their average. In addition, a one-way ANOVA was done to compare the data of cardiorespiratory responses, StO_2 , and power output at VT as detected by both methods. An a posteriori Fisher's procedure for learning systems design (PLSD) test was used when the F-ANOVA ratio was significant. A linear regression analysis was performed to determine relationships between $VT_{V-slope}$ and VT_{nirs} for each parameter. Statistical analyses were done using statistical software (StatView, SAS Institute, and MedCalc 7.2). All data are reported as means and standard deviation ($\pm$ SD). The level of significance was fixed at $p < 0.05$.

Results

The mean and standard deviation of anthropometric characteristics, spirometric parameters, as well as cardiorespiratory responses and the power output at peak exercise are presented in Table 1. Pulmonary function was normal and no restrictive or ob-

Fig. 1a to c Typical example of the VT determination for one subject by $VT_{V-slope}$ (a upper graph), and VT_{nirs} (b middle graph). The lower graph (c) showed StO_2 as a function of $\dot{V}E$ which reflects mechanical load of the respiratory muscles.

structive syndrome was observed. All exercise tests performed by children conformed to criteria for maximal effort, except for two subjects stopping the test prematurely; thus we excluded them from study.

Oxygenation changes

A typical trace of StO_2 during incremental exercise for one subject is given in Fig. 2. After a rest period, a slight increase in the NIRS signal during the warm-up period was observed. Thereafter, StO_2 decreases progressively until a rapid fall and abrupt decrease of the oxygenation graph was observed. This breakpoint in the curve linearity corresponds to VT_{nirs} . During recovery, a fast increase in StO_2 which got above the resting value (hyperemia phase) was noted.

Relationship between $VT_{V-slope}$ and VT_{nirs}

To validate the detection of VT by NIRS on the respiratory muscle we have assessed the relationship and the agreement between the $VT_{V-slope}$ and VT_{nirs} methods. The comparisons between parameters at VT detected by these methods are listed in Table

Table 1 Anthropometric and spirometric parameters, and peak exercise results in children

Subjects (n = 12)		Value
Anthropometric		
– age	(yr)	12.8 ± 1.4
– height	(cm)	155.1 ± 8.7
– weight	(kg)	46.4 ± 7.7
– BMI	(kg/m ²)	19.1 ± 1.9
Spirometric		
– FEV1	(l)	2.69 ± 0.43
	(%pred)	100.05 ± 14.28
– FVC	(l)	3.21 ± 0.55
	(%pred)	93.01 ± 13.52
– TLC	(l)	4.25 ± 0.61
	(%pred)	90.56 ± 13.06
– FEV1/FVC ratio		1.04 ± 0.16
– MVV	(l/min)	107.47 ± 17.35
Peak exercise		
– power output	(W)	156.60 ± 27.8
– $\dot{V}O_{2max}$	(ml/min/kg)	47.50 ± 6.3
	(l/min)	2.20 ± 0.46
– $\dot{V}CO_{2max}$	(l/min)	2.37 ± 0.46
– $\dot{V}E_{max}$	(l/min)	76.40 ± 12.2
– HR_{max}	(beats/min)	196.0 ± 11

* Values are means ± SD. BMI: body mass index; FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; TLC: total lung capacity; %pred: percentages of predicted values

2. No significant differences were observed between parameters determined by both $VT_{V-slope}$ and VT_{nirs} ($p > 0.05$).

Analysis of linear regression between $VT_{V-slope}$ and VT_{nirs} for each parameter is shown in Fig. 3 (left panels). We found a strong positive correlation between the two methods ($p < 0.001$) for all variables. Correlation coefficients for $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$ were $r = 0.93$ ($p < 0.0001$), $r = 0.94$ ($p < 0.001$), and $r = 0.87$ ($p < 0.002$), respectively. The correlation coefficients for StO_2 and power out-

Table 2 Comparison between parameters at ventilatory threshold detected by both NIRS and V-slope

Parameters		VT_{nirs}	$VT_{V-slope}$	p-value
$\dot{V}E$	(l/min)	43.4 ± 7.0	40.5 ± 8.5	(NS)
	(% of max)	56.4 ± 11.1	52.2 ± 10.7	
$\dot{V}O_2$	(ml/min/kg)	34.8 ± 6.2	33.4 ± 6.7	(NS)
	(% of max)	73.8 ± 6.9	69.7 ± 6.8	
$\dot{V}CO_2$	(l/min)	1.48 ± 0.32	1.38 ± 0.39	(NS)
	(% of max)	62.6 ± 7.8	57.6 ± 7.2	
Power output (W)		103.7 ± 21.1	96.6 ± 27.6	(NS)
HR (beats/min)		153.0 ± 10	147.0 ± 4	(NS)
StO_2 (%)		36.3 ± 7.4	37.8 ± 7.1	(NS)

put were $r = 0.94$ ($p < 0.0001$) and $r = 0.89$ ($p < 0.0001$), respectively. The data detected by both methods were sometimes similar; for this reason, some values were superimposed on the graph, and the number of points is different than 12 (number of the subjects interpreted).

Fig. 3 (right panels) contains the Bland-Altman plots illustrating the mean differences (± 1.96 SD) against the average value obtained by both $VT_{V-slope}$ and VT_{nirs} for $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$. Overall, the difference in scores was within 95% of the confidence interval, showing that both methods agree closely. The 95% limits of agreement were therefore from -0.12 to 0.24 l/min, -0.18 to 0.38 l/min, and -5.2 to 11.2 l/min for $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$, respectively.

Discussion

This study was performed to assess respiratory muscle oxygenation and to validate the use of NIRS as an additional method for the detection of VT in children. Two major findings were observed. Firstly, the respiratory muscles revealed by NIRS deoxygenate during incremental exercise in children; secondly, com-

Fig. 2 Schematic representation of an NIRS measurement during incremental exercise. The values obtained between minimal (0%, end of exercise) and maximal oxygenation (100%, hyperemia) indicate changes in muscle oxygenation during exercise.

Fig. 3 Left panels contain the relationship between $VT_{V-slope}$ and VT_{NIRS} for an entire population. Right panels show the Bland-Altman plots il-

lustrating the mean differences (± 1.96 SD) between the $VT_{V-slope}$ and VT_{NIRS} methods versus their average.

pared to V-slope, NIRS could be used as a valid tool in the determination of VT.

During incremental exercise test, the cardiorespiratory responses at peak exercise and at VT were in agreement with the results of previous investigations within the range for similar children [15,21,29]. In the same way, lung function tests demonstrated normal and standard and no respiratory diseases were detected. The end point of an exercise test is greatly influenced by the motivation of the subject, particularly for children [1]. To avoid methodological interpretations of our results, two subjects

were excluded because peak exercise was not reached and/or VT data were suspect.

To date, the majority of the studies evaluating tissue oxygenation by NIRS have been interested in peripheral muscles, particularly the vastus lateralis muscle [12,13]. In the present study, we investigated oxygenation of the respiratory muscles because their metabolism can be affected, which consequently alters exercise performance [2,20]. In fact, tests and measurements of respiratory muscles are not performed routinely, and development of noninvasive techniques such as occlusion pressure, tension-time index, surface electromyography (EMG), and NIRS permits a me-

ticulous comprehension of respiratory function response and adaptation. The most important muscle of inspiration is the diaphragm but various other muscles such as intercostals, scalenes, triangularis, sternocleidomastoid, and pectoralis contribute additionally to inspiration. In the current study, we focused on an accessory inspiratory muscle, the anterior serratus, for two reasons: primarily, it is difficult to have access to the diaphragm muscle because of its anatomical arrangement; secondly, the anterior serratus muscle attach to the rib cage at an extrathoracic anchoring point, the shoulder girdle. Thus, under conditions of increased ventilatory demand, this muscle plays a major role in the increase of ventilation [16]. In the literature, the term respiratory muscles are used to avoid confusion. In this sense, Gaultier [18] concluded that it is more appropriate to describe the values obtained when recording diaphragm activity by surface electrode EMG as chest wall EMG rather than diaphragmatic EMG because tracings can be contaminated by other inspiratory or expiratory muscles.

The originality of our work was the use of NIRS in the assessment of respiratory muscles oxygenation. During exercise, StO_2 decreased progressively as exercise intensity increased. This result indicates a reduced oxygen transport to the respiratory muscles, which causes hypoxia [2]. The NIRS signal coming from the capillaries, arterioles, and venules, reflects the balance between local muscle oxygen delivery and muscle oxygen utilization at the site of oxygen exchange within the region of NIRS interrogation [9]. Therefore, the decreased signal shown in our study indicates a decrease in oxygenation, i.e., impaired oxygen utilization by respiratory muscles because of insufficient oxygen supply to support contractile activity. Flaminiano and Celli [16] and Jonville et al. [20] have reported that the diaphragm function depends directly on the ability of the circulation to deliver adequate oxygen and substrates. Our results agree with the findings of Mancini et al. [24] and Terekado et al. [33] who reported deoxygenation of respiratory muscles associated with greater work of breathing during exercise in normal subjects and patients with heart failure. Unfortunately, no pediatric study reported oxygenation states in children, but compared to adults, they present the same cardiovascular responses [10,30,34] and the same respiratory muscle pressures [18] relative to their body size. In contrast, children are characterized by shallow and rapid pulmonary ventilation and a higher respiratory rate [21].

Detection of VT from the dynamic responses of both gas exchange and StO_2 data during incremental exercise using $\text{VT}_{\text{V-slope}}$ and VT_{nirs} has shown high relationship, and significant correlation ($r=0.87$ to 0.94) was found between these two methods. VT is observed when muscle oxygen requirements begin to exceed that provided, which causes as a consequence an increase in the expired CO_2 generated from the bicarbonate buffering of lactic acid [36]. In our study, Fig. 1c illustrated the decrease of oxygenation and the increase of ventilation reflecting thus perfectly the mechanical load of the respiratory muscle during exercise. The correlations obtained between $\text{VT}_{\text{V-slope}}$ and VT_{nirs} indicate that the anaerobic metabolism solicitation occurs simultaneously in both respiratory muscles and peripheral muscles as a result of deoxygenation. However, Bhambhani et al. [6] have found that VT detected by NIRS in peripheral muscle (vastus lateralis) coincides with VT found by the V-slope method in adults.

The metabolism adaptation, of course, depends on oxygen, i.e., blood flow which supplies energy substrates and washes out metabolites that can impair muscle contractility [35]. Since the distribution of cardiac output during exercise to specific tissues is generally proportional to their metabolic activity, Harms [19] concluded that exercise performance may also be affected by the work of breathing during heavy exercise due to the redistribution of blood flow between the chest wall and the locomotor muscles. In the present investigation, we compared the difference in data between the V-slope and NIRS methods of VT determination, and we observed clearly a good agreement, as graphically shown in Bland-Altman plots (Fig. 3). In fact, the difference scores (expressed as mean and SD) were centered closely around zero, and all data were inside the ± 1.96 SD fixed interval. Thus, the strong relationship between $\text{VT}_{\text{V-slope}}$ and VT_{nirs} demonstrate that NIRS permits the detection of the VT of respiratory muscles in children. Nevertheless, the difficulty of such graphical method arises when there is no clear breaking point from linearity in the curve. For this reason the visual determination of VT from the graph was considered a subjective judgment [7], but the use of experienced and independent observers excludes this hypothesis.

In summary, this study demonstrates that respiratory muscles deoxygenate during incremental exercise in children. Thus, the findings suggest that restricted oxygen delivery could contribute to the onset of anaerobic metabolism and probably fatigue of respiratory muscles. Furthermore, this investigation showed a close agreement between $\text{VT}_{\text{V-slope}}$ and VT_{nirs} and validates the use of NIRS as an additional tool for the determination of VT. In perspective it would be interesting to use VT of respiratory muscle as an index of efficiency, and as an intensity of training in healthy or patient subjects.

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