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Using a portable near-infrared spectroscopy device to estimate the second ventilatory threshold

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1 **USING A PORTABLE NEAR-INFRARED SPECTROSCOPY DEVICE TO**
2 **ESTIMATE THE SECOND VENTILATORY THRESHOLD**
3

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ABSTRACT

The aim was to examine the association between this breakpoint (NIRS) and VT_2 in well-trained runners. Gas exchange and NIRS data were collected during an incremental VO_{2max} running test for 10 well-trained runners. The breakpoint calculated in oxygen saturation (StO_2) and the VT_2 were determined and compared in terms relative to $\%VO_{2max}$, absolute speed, VO_2 and maximum heart rate (HR_{max}). There were no significant differences ($p>0.05$) between the breakpoint in StO_2 and VT_2 relative to $\%VO_{2max}$ (87.00 ± 6.14 and 88.28 ± 3.98 %), absolute speed (15.70 ± 1.42 and 16.10 ± 1.66 $km\cdot h^{-1}$), VO_2 (53.71 ± 15.17 and 54.66 ± 15.57 $ml\cdot kg^{-1}\cdot min^{-1}$) and $\%HR_{max}$ (90.90 ± 4.17 and 91.84 ± 3.70 %). There were large and significant correlations between instruments relative to $\%VO_{2max}$ ($r=0.68$, $p<0.05$), absolute speed ($r=0.86$, $p<0.001$), VO_2 ($r=0.86$, $p<0.001$) and $\%HR_{max}$ ($r = 0.69$; $p<0.05$). A Bland and Altman analysis of agreement between instruments resulted in a mean difference of $-1.27\pm4.49\%$, -0.40 ± 0.84 $km\cdot h^{-1}$, -0.90 ± 3.07 $ml\cdot kg^{-1}\cdot min^{-1}$, and -0.94 ± 3.14 for $\%VO_{2max}$, absolute speed, VO_2 and $\%HR_{max}$, respectively. We conclude that a portable NIRS determination of the StO_2 breakpoint is comparable with VT_2 using gas exchange and therefore appropriate for use in determining exercise training above VT_2 intensity. This is the first study to analyze the validity with the running mode using a NIRS portable device.

Keywords: intramuscular oxygenation, gas exchange threshold, incremental running test, athletes, exercise intensity

INTRODUCTION

Quantification of training intensity according to different physiological parameters such as heart rate (HR), blood lactate ($[La^-]$) or maximal oxygen consumption (VO_{2max}) has been used to individualize training prescription to improve performance [1]. Several physiological thresholds (ventilatory and lactate thresholds, critical power) have been determined and used to prescribe training zones [2]. Often, training intensity relative to VO_{2max} ($\%VO_{2max}$), or HR ($\%HR$), is used to differentiate intensity zones [2]. However, recent studies [3,4] have determined first and second ventilatory turn-points or lactate thresholds (2 to 4 mM blood lactate concentration range) to demarcate three intensity zones (zone 1, zone 2 and zone 3).

The prescription of training intensity by ventilatory or lactate thresholds typically requires expensive equipment (gas analysers, treadmill or cycle ergometer and blood lactate analyser) and several laboratory visits. Athletes are often hesitant to have repeated venipunctures of fingers or earlobes for blood collection or find breath-by-breath gas exchange obtrusive [5]. Therefore, coaches and practitioners need good, wearable and low-cost instruments that provide physiological responses which can deliver real-time feedback to athletes [6]. In addition, HR, $[La^-]$, and VO_{2max} are indicative of systemic changes of the body, with no specific information about the working muscles. Thus, a non-invasive method of determining training zones and local changes (muscles) would be beneficial.

Near-infrared spectroscopy (NIRS) has recently been used to determine total muscle oxygen saturation at rest and during exercise in a variety of anatomical positions and contexts [7,8]. Interest in examining muscle oxygen saturation (StO_2) has been growing using these devices (Lab settings and wearable), measuring hemoglobin oxygenation in tissue using spectrophotometric principles [5,6,9].

Snyder and Parmenter [5] reported that near-infrared spectroscopy determination of a breakpoint in percent oxygen saturation is a noninvasive technique that is comparable with blood lactate concentration in determining maximal lactate steady state intensity and therefore appropriate for use in determining exercise training intensity.

The validity of NIRS devices has been evident in cycling but not in running. Fontana et al. [1] affirmed that during incremental cycling exercise in 118 healthy men, the VO_2 associated with the onset of a plateau in NIRS-derived deoxygenated hemoglobin (deoxy[Hb]) occurs in coincidence with the VO_2 at the respiratory compensation point (RCP). They suggested that this breakpoint in the ventilation response can be accurately estimated, using NIRS-derived measures of deoxygenated hemoglobin as an alternative to the use of ventilatory or blood-based techniques. Iannetta et al.,[9] indicated that the deoxy[Hb] concentration measured by near-infrared spectroscopy is a repeatable measure during ramp incremental cycling tests, associated with pulmonary oxygen uptake breaking-point corresponding to the RCP. Farzam, Starkweather, Franceschini [6] compared the oxygen saturation and haemoglobin/myoglobin concentration in the exercising muscles of 17 athletic individuals during cycling measured simultaneously with two systems (NIRS system with use limited to lab settings vs. wearable NIRS [Humon HEX]). They demonstrated correlation coefficients of the StO_2 and haemoglobin/myoglobin concentrations between the two systems of over 0.70 during exercise, and that NIRS values also showed a good accuracy in predicting the lactate threshold level.

However, relatively little is still known about the validity and reliability of these wearable devices, because there are a small number of wearables, fiberless, NIRS devices used in the athletic market, and they are as yet not frequently used by coaches and practitioners. Only the study of Farzam, Starkweather, Franceschini [6] reported the reliability of a

portable NIRS device (Humon HEX) in cycling, but the validity and reliability of this wearable device is currently unknown during running. Therefore, this study aimed to determine whether NIRS (Humon HEX) could detect a breakpoint in StO₂ of the muscle and whether the exercise intensity (%VO_{2max}, absolute speed, VO₂ relative to body mass and %HRmax) could be used to predict VT₂ during an incremental running VO_{2max} test in well-trained runners. We hypothesized that the wearable NIRS (Humon HEX) device would be reliable to predict VT₂ during an incremental running VO_{2max} test.

MATERIALS AND METHODS

Participants

Ten (five men and five women) well-trained runners (mean $\pm$ SD: 21.22 $\pm$ 1.30 years; VO_{2max} 66.40 $\pm$ 8.28 ml·kg⁻¹·min⁻¹; 56.57 $\pm$ 9.11 kg and 166.45 $\pm$ 7.86 cm) volunteered to participate in this study. All participants were experienced middle- and long-distance runners free from injury within the previous six months. Prior to the study, all participants were informed about the testing protocols, possible risks involved and invited to provide written informed consent. The study was performed in accordance with the principles of the Declaration of Helsinki (October 2008, Seoul), and the experimental protocols were approved by the local ethics committee. This study meets the ethical standards outlined by the International Journal of Sports Medicine [10].

Experimental design

Participants visited the laboratory (550m altitude, 20-25°, 35-40% relative humidity) in a non-fatigued state (no intense exercise in the previous 48h) and completed an incremental maximal running test on a treadmill (HP Cosmos Pulsar, HP Cosmos Sports & Medical GMBH, Nussdorf-Traunstein, Germany). The test started at 2.5 m·s⁻¹ for 5-min (warm-

up). Then, the speed increased by $0.28 \text{ m}\cdot\text{s}^{-1}$ every minute until volitional exhaustion. The treadmill slope was kept at 1% to imitate external wind conditions [11,12]. During the test, respiratory variables were measured using a gas analyzer (CPX Ultima Series MedGraphics, St. Paul, Minnesota, USA), which was calibrated prior to each session (CO_2 4.10%; O_2 15.92%). The exercise heart rate (HR) of the participants were monitored and recorded at the end of each stage. The average VO_2 value obtained during the last 30s of the final running stage was considered as $\text{VO}_{2\text{max}}$ when at least two of the following criteria were fulfilled [13]: (1) a plateau in VO_2 (an increase of less than $1.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in two consecutive workloads; (2) Respiratory exchange ratio (RER) >1.15 ; (3) maximal HR values above 95% of the age-predicted maximum (220-age). The average of oxygen uptake values during the last 30 s in the final stage of the incremental test was considered as $\text{VO}_{2\text{max}}$, because there is considerable breath-by-breath variability in pulmonary gas exchange (PGE), the average of the PGE values over 30 s periods is very effective during incremental exercise tests [14].

The second ventilatory threshold (VT_2) was identified by the nonlinear increase in VE/VCO_2 curve concomitant to a second strong increase in VE/VO_2 , with a further increase in exercise intensity [15], confirmed with a decrease in partial pressure of PetCO_2 or VCO_2 slope [16].

A portable NIRS (near infrared spectroscopy) device, the Humon HEX [6] was placed on the vastus lateralis belly approximately halfway between the trochanter and the knee joints of the subject's right leg [9,17] and secured in place by an elastic strap and by an elastic band to minimize movement. The device contains six slots through which it emits LED radiation at different wavelengths (760-840nm), used to determine the haemoglobin content, its saturation in g/dL and percentage. The measurement is based on the different absorbance wavelengths of haemoglobin and oxyhaemoglobin. The concentration of

deoxyhaemoglobin can then be calculated with the formula $[\text{deoxyhaemoglobin (g/dL)} = \text{haemoglobin (g/dL)} - \text{oxyhaemoglobin (g/dL)}]$. Data were stored online at an output frequency of 1 Hz. The device was previously calibrated following manufacturer recommendations.

During an incremental test, the behaviour of oxyhaemoglobin (oxy[Hb]) has been described as a double-linear function, exhibiting a breaking point representing the attenuation of the increase in oxy[Hb] and the onset of a plateau-like response [9].

Changes in the absorption of deoxygenated haemoglobin deoxy[Hb] and oxy[Hb] allow the calculation of the percentage of oxygen saturation (StO₂) in the tissue [5]. Therefore, the method for identifying the breakpoint in StO₂ was initially the visual identification of the workload before a prolonged decrease of StO₂ that lead to a continuous decrease [18,19]. A decrease of more than 15% of StO₂ was used as the criterion to confirm the sustained nature of the reduction in StO₂ for all subjects [5].

In this way, the breakpoint in StO₂ and the VT₂ were detected individually for each of the subjects by means of an observation method following the criteria mentioned above for each variable. The second-by-second data were then aligned in time so that the time "zero" represented the beginning of the exercise. In this way, the values of both measurement methods could be checked by two experimented researchers (FGM and JMGR), with disagreement settled by consensus (Figure 1).

<Figure 1 about here>

Statistical analysis

The results are expressed as means and standard deviation (SD). The Shapiro-Wilk test was used to check the homogeneity of each variable ($p > 0.05$). First, paired t-tests were used to compare the breakpoint in StO₂ and VT₂ with the two different methods

established (NIRS and gas exchange). Secondly, a Pearson's product-moment correlation coefficient (r) was calculated to establish the association between both markers. Finally, a Bland and Altman analysis [20] was performed to examine the agreement between the criterion $\%VO_{2max}$, the speed, HR and VO_2 predicted by breakpoint in StO_2 and VT_2 and the systematic bias, standard deviations and the homoscedasticity/heteroscedasticity of the data, which was calculated as the correlation between absolute differences and means [21]. The level of statistical significance was set at $P \leq 0.05$.

Results

The individual and group results for breaking in StO_2 and VT_2 are shown in Table 1.

<Table 1 about here>

There were no significant differences ($p = 0.39$, $ES = 0.28$) between the breakpoint in StO_2 expressed relative to VO_{2max} ($\%VO_{2max}$) ($86.99 \pm 6.14\%$) compared to VT_2 ($88.27 \pm 3.98\%$). In addition, there were no significant differences ($p = 0.17$, $ES = 0.47$) between the breakpoint in StO_2 ($15.70 \pm 1.42 \text{ km}\cdot\text{h}^{-1}$) compared to VT_2 ($16.10 \pm 1.66 \text{ km}\cdot\text{h}^{-1}$) when expressed as absolute speeds. There were no significant differences ($p = 0.34$, $ES = 0.32$) between the breakpoint in StO_2 ($53.71 \pm 15.17 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) compared to VT_2 ($54.66 \pm 15.57 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) when expressed as VO_2 relative to body mass. Finally, when it was expressed as $\%HR_{max}$, there were no significant differences ($p = 0.37$, $ES = 0.30$) between the breakpoint of StO_2 ($90.90 \pm 4.17 \%$) compared to VT_2 ($91.84 \pm 3.70 \%$). Pearson's product-moment correlation coefficients revealed significant associations between the breakpoint in StO_2 and VT_2 when expressed relative to VO_{2max} ($\%VO_{2max}$) ($r = 0.68$, $SEE = 4.75\%$, $p < 0.05$), absolute running speed ($r = 0.86$, $SEE = 0.76\%$, $p < 0.001$), VO_2 relative to body mass ($r = 0.86$, $SEE = 2.85\%$, $p < 0.001$) and $\%HR_{max}$ ($r = 0.69$; $SEE = 3.21\%$, $p < 0.05$).

Finally, the Bland and Altman analysis of agreement between the breakpoint in StO₂ and VT₂ relative to %VO_{2max} resulted in a mean difference of -1.27 ± 4.49 % (limits of agreement: lower = -10.08; upper = 7.54%), whereas the mean difference relative to the speed was -0.40 ± 0.84 km·h⁻¹ (limits of agreement: lower = -2.05; upper = 1.25 km·h⁻¹) and relative to the VO₂ was -0.90 ± 3.07 ml·kg⁻¹·min⁻¹ (limits of agreement: lower = -6.99; upper = 5.04 ml·kg⁻¹·min⁻¹) (Figure 2). There was no evidence of systematic bias between the two methods. The analysis of correlation of the absolute difference between instruments means in the Bland-Altman plots showed no heteroscedasticity for the variables analyzed ($r = 0.22, 0.27, 0.09$ and 0.03 when expressed as VO₂ relative to body mass, relative to VO_{2max} [%VO_{2max}], absolute running speed and %HRmax, respectively).

<Figure 2 about here>

Discussion

The purposes of this study were to determine whether a portable NIRS device (Humon HEX) could detect a breakpoint in StO₂ of the muscle during an incremental running VO_{2max} test and whether the relative exercise intensity (%VO_{2max}), absolute speed, HR and VO₂ at this breakpoint in StO₂ could be used to predict VT₂ exercise intensity in well-trained runners. To our knowledge, this is the first study that shows an association between breakpoint in StO₂ with a portable NIRS device (Humon HEX) and VT₂ in runners.

The first finding was that there were no significant differences between the breakpoint in StO₂ and VT₂ relative to %VO_{2max}, at absolute speed (km·h⁻¹), VO₂ relative to body mass nor %HRmax. Secondly, the breakpoint in StO₂ and VT₂ were strongly correlated when expressed relative to VO_{2max} ($r = 0.68$), at the absolute speed ($r = 0.86$), VO₂ relative to body mass ($r = 0.86$) and %HRmax ($r = 0.69$) during the incremental VO_{2max} test. In this

way, the exercise intensity (relative and absolute) predicted by the breakpoint with Humon HEX and VT_2 were similar. Our results showed a standard error of the estimate (SEE) between both breakpoint in StO_2 and VT_2 of 4.75%, 0.76%, 2.85% and 0.99% when expressed relative to VO_{2max} , absolute speed, VO_2 relative to body mass and %HRmax, respectively.

Our results are in accordance with previous studies that found an association between breakpoint in StO_2 and the respiratory compensation point (also referred as VT_2) during cycle ergometer exercise in well-trained men and women [9] and healthy men [1]. Importantly, running on a treadmill involves considerably more movement and impact than cycling, yet these data show that this technique can still be successfully used in trained runners on a treadmill.

Muscle oxygenation can guide athletes' training by examining how their muscles are responding to exertion and recovery. In incremental exercise, muscle oxygenation exhibits three phases: a small adjustment at the beginning of the test due to the effect of muscle contraction; a greater increase in deoxy[Hb] due to a greater extraction of oxygen and finally, a plateau due to a lesser dependence on oxygen by an anaerobic phase [6,17].

The scientific literature explains possible causes that influence the plateau, the most important is the vasodilation and redistribution of blood flow to the active muscle [1,9] and a greater contribution from non-oxidative sources along with the recruitment of type II fibres [1,17]. In addition, deoxy[Hb] could define the boundary between heavy and very heavy/severe domains (VO_2 and [La] can no longer stabilize). Another study found a similar value for the VO_2 associated with the breakpoint, RCP and MLSS, highlighting that those variables may share similar mechanistic bases [22].

In order to quantify the exercise intensity athletes and coaches rely on measurements of heart rate, blood lactate concentration or maximum oxygen uptake (VO_{2max}) [23,24].

227 However, athletes and coaches require portable devices that they can use in the field, with
228 instantaneous data and better information. The breakpoint with Humon HEX provides
229 much more information compared to just looking at the heart rate, and the data acquisition
230 of endurance sessions allows a better monitorization of a physiological response which
231 can deliver real-time feedback to athletes [6]. Farzam, Starkweather, Franceschini,[6]
232 validated the performance of a low-cost, wireless, wearable NIRS (Humon HEX) device
233 against an advanced benchtop device, during an incremental cycling test with 17 healthy
234 men. Our findings confirm the level of agreement between the two measurement systems
235 (NIRS vs. VT_2) with the Bland-Altman plot. According to our results, similar correlations
236 were found between breakpoint in StO_2 and VT_2 using $\%HR_{max}$ and $\%VO_{2max}$.
237 Therefore, it would be interesting monitoring training sessions using Humon HEX device
238 along with $\%HR_{max}$ as a valid reference by coaches.

239 However, the major drawback of the Humon HEX device is the lack of information about
240 the exact point at which the athlete reaches VT_1 . It is also worth noting that the Humon
241 HEX App provided a simple method in the dashboard for determining VT_2 , but it cannot
242 instantaneously be provided to athletes with detailed intensities relative to VO_{2max} by
243 indicating different physiological intensities using colours on the screen (e.g., blue: rest;
244 red: above VT_2). In contrast, a strength of the approach using the breakpoint is that it is
245 more stable than the threshold detected by blood lactate [6], because muscle oxygenation
246 responses during exercise tend to have a stable pattern [9,25]. As an acute response, at
247 the beginning of the exercise, muscle oxygen saturation increases slightly. From here, as
248 the intensity of the exercise increases, the saturation begins to decrease progressively
249 without suffering any turning points. Therefore, NIRS may be a more applicable and safer
250 method of detecting thresholds during exercise than using lactate concentrations, in
251 addition to the obviously less invasive data measurement.

We recognise that the results obtained in this study are limited to the specific participants and the low number of runners; future studies with larger cohorts should be undertaken to confirm these results. Another possible limitation was that factors such as fat tissue thickness, skin temperature and muscle fiber density that can affect the breakpoint of could not be controlled [5]. However, all tests were performed on the same area of muscle to reduce the effect of these factors. In addition, these results must be carefully considered due to differences between the measure of VT_2 by breath-by-breath or absolute speed and breakpoint of StO_2 by the wearable NIRS device (Humon HEX) because the limits of agreement are high (Figure 2).

In conclusion, this study validates the performance of a low cost, wireless, wearable NIRS device against VT_2 obtained by gas exchange in an incremental maximal running test on a treadmill, confirming the consistency of values reported and an acceptable level of agreement between the two measurement systems. The NIRS device can be a useful tool for coaches and runners to easily and effectively measure the breakpoint of StO_2 and thus be able to determine training zones above VT_2 . It can also be used to evaluate improvements in aerobic capacity without the use of complex and more expensive oxygen uptake methods, as well as for analysis in the field.

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Figure legends

Figure 1. Example of determination of breakpoint in StO₂ and VT₂.

Figure 2. Bland-Altman plots for the Humon HEX devise and VT₂ in VO₂ relative to body mass (A), km·h⁻¹ (B), %VO_{2max} (C) and %HR_{max} (D). The central line represents the absolute average difference between instruments, the upper and lower lines represent ± 1.96 standard deviations (SD), while the gray line is the regression line of the data points and the curved line represents its 95%CI.

Table legends

Table 1. Descriptive data for the study variables and results between breakpoint in StO₂ and VT₂ relative to VO_{2max}, absolute speed, HRmax and VO₂.