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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

Preferred running head: NIRS estimated ventilatory threshold in runners

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5

6 **ABSTRACT**

7 The aim was to examine the association between this breakpoint (NIRS) and VT_2 in well-
8 trained runners. Gas exchange and NIRS data were collected during an incremental
9 VO_{2max} running test for 10 well-trained runners. The breakpoint calculated in oxygen
10 saturation (StO_2) and the VT_2 were determined and compared in terms relative to
11 $\%VO_{2max}$, absolute speed, VO_2 and maximum heart rate (HR_{max}). There were no
12 significant differences ($p>0.05$) between the breakpoint in StO_2 and VT_2 relative to
13 $\%VO_{2max}$ (87.00 ± 6.14 and 88.28 ± 3.98 %), absolute speed (15.70 ± 1.42 and 16.10 ± 1.66
14 $km\cdot h^{-1}$), 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
15 and 91.84 ± 3.70 %). There were large and significant correlations between instruments
16 relative to $\%VO_{2max}$ ($r=0.68$, $p<0.05$), absolute speed ($r=0.86$, $p<0.001$), VO_2 ($r=0.86$,
17 $p<0.001$) and $\%HR_{max}$ ($r = 0.69$; $p<0.05$). A Bland and Altman analysis of agreement
18 between instruments resulted in a mean difference of $-1.27\pm 4.49\%$, -0.40 ± 0.84 $km\cdot h^{-1}$, -
19 0.90 ± 3.07 $ml\cdot kg^{-1}\cdot min^{-1}$, and -0.94 ± 3.14 for $\%VO_{2max}$, absolute speed, VO_2 and $\%HR_{max}$,
20 respectively. We conclude that a portable NIRS determination of the StO_2 breakpoint is
21 comparable with VT_2 using gas exchange and therefore appropriate for use in determining
22 exercise training above VT_2 intensity. This is the first study to analyze the validity with
23 the running mode using a NIRS portable device.

24

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

27 INTRODUCTION

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

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

47 Near-infrared spectroscopy (NIRS) has recently been used to determine total muscle
48 oxygen saturation at rest and during exercise in a variety of anatomical positions and
49 contexts [7,8]. Interest in examining muscle oxygen saturation (StO₂) has been growing
50 using these devices (Lab settings and wearable), measuring hemoglobin oxygenation in
51 tissue using spectrophotometric principles [5,6,9].

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

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

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

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

84

85 **MATERIALS AND METHODS**

86 *Participants*

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

96

97 *Experimental design*

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

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

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

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

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

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

134 Changes in the absorption of deoxygenated haemoglobin deoxy[Hb] and oxy[Hb] allow
135 the calculation of the percentage of oxygen saturation (StO₂) in the tissue [5]. Therefore,
136 the method for identifying the breakpoint in StO₂ was initially the visual identification of
137 the workload before a prolonged decrease of StO₂ that lead to a continuous decrease
138 [18,19]. A decrease of more than 15% of StO₂ was used as the criterion to confirm the
139 sustained nature of the reduction in StO₂ for all subjects [5].

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

146 <Figure 1 about here>

147

148 *Statistical analysis*

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

152 established (NIRS and gas exchange). Secondly, a Pearson's product-moment correlation
153 coefficient (r) was calculated to establish the association between both markers. Finally,
154 a Bland and Altman analysis [20] was performed to examine the agreement between the
155 criterion $\%VO_{2max}$, the speed, HR and VO_2 predicted by breakpoint in StO_2 and VT_2 and
156 the systematic bias, standard deviations and the homoscedasticity/heteroscedasticity of
157 the data, which was calculated as the correlation between absolute differences and means
158 [21]. The level of statistical significance was set at $P \leq 0.05$.

159

160 **Results**

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

162 <Table 1 about here>

163 There were no significant differences ($p = 0.39$, $ES = 0.28$) between the breakpoint in
164 StO_2 expressed relative to VO_{2max} ($\%VO_{2max}$) ($86.99 \pm 6.14\%$) compared to VT_2 (88.27
165 $\pm 3.98\%$). In addition, there were no significant differences ($p = 0.17$, $ES = 0.47$) between
166 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
167 expressed as absolute speeds. There were no significant differences ($p = 0.34$, $ES = 0.32$)
168 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 ($54.66 \pm$
169 $15.57 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) when expressed as VO_2 relative to body mass. Finally, when it was
170 expressed as $\%HR$ max, there were no significant differences ($p = 0.37$, $ES = 0.30$)
171 between the breakpoint of StO_2 ($90.90 \pm 4.17 \%$) compared to VT_2 ($91.84 \pm 3.70 \%$).

172 Pearson's product-moment correlation coefficients revealed significant associations
173 between the breakpoint in StO_2 and VT_2 when expressed relative to VO_{2max} ($\%VO_{2max}$)
174 ($r = 0.68$, $SEE = 4.75\%$, $p < 0.05$), absolute running speed ($r = 0.86$, $SEE = 0.76\%$, $p <$
175 0.001), VO_2 relative to body mass ($r = 0.86$, $SEE = 2.85\%$, $p < 0.001$) and $\%HR_{max}$ ($r =$
176 0.69 ; $SEE = 3.21\%$, $p < 0.05$).

177 Finally, the Bland and Altman analysis of agreement between the breakpoint in StO₂ and
178 VT₂ relative to %VO_{2max} resulted in a mean difference of -1.27 ± 4.49 % (limits of
179 agreement: lower = -10.08; upper = 7.54%), whereas the mean difference relative to the
180 speed was -0.40 ± 0.84 km·h⁻¹ (limits of agreement: lower = -2.05; upper = 1.25 km·h⁻¹)
181 and relative to the VO₂ was -0.90 ± 3.07 ml·kg⁻¹·min⁻¹ (limits of agreement: lower = -
182 6.99; upper = 5.04 ml·kg⁻¹·min⁻¹) (Figure 2). There was no evidence of systematic bias
183 between the two methods. The analysis of correlation of the absolute difference between
184 instruments means in the Bland-Altman plots showed no heteroscedasticity for the
185 variables analyzed ($r = 0.22, 0.27, 0.09$ and 0.03 when expressed as VO₂ relative to body
186 mass, relative to VO_{2max} [%VO_{2max}], absolute running speed and %HRmax, respectively).

187 <Figure 2 about here>

188

189 **Discussion**

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

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

202 way, the exercise intensity (relative and absolute) predicted by the breakpoint with
203 Humon HEX and VT_2 were similar. Our results showed a standard error of the estimate
204 (SEE) between both breakpoint in StO_2 and VT_2 of 4.75%, 0.76%, 2.85% and 0.99%
205 when expressed relative to VO_{2max} , absolute speed, VO_2 relative to body mass and
206 %HRmax, respectively.

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

213 Muscle oxygenation can guide athletes' training by examining how their muscles are
214 responding to exertion and recovery. In incremental exercise, muscle oxygenation
215 exhibits three phases: a small adjustment at the beginning of the test due to the effect of
216 muscle contraction; a greater increase in deoxy[Hb] due to a greater extraction of oxygen
217 and finally, a plateau due to a lesser dependence on oxygen by an anaerobic phase [6,17].
218 The scientific literature explains possible causes that influence the plateau, the most
219 important is the vasodilation and redistribution of blood flow to the active muscle [1,9]
220 and a greater contribution from non-oxidative sources along with the recruitment of type
221 II fibres [1,17]. In addition, deoxy[Hb] could define the boundary between heavy and
222 very heavy/severe domains (VO_2 and [La] can no longer stabilize). Another study found
223 a similar value for the VO_2 associated with the breakpoint, RCP and MLSS, highlighting
224 that those variables may share similar mechanistic bases [22].

225 In order to quantify the exercise intensity athletes and coaches rely on measurements of
226 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.

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

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

269

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273

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348

Figure legends

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

Figure 2. Bland-Altman plots for the Humon HEX device 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.