



A proposal to identify the maximal metabolic steady state by muscle oxygenation and VO_2 max levels in trained cyclists

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Abstract

Purpose Near-infrared spectroscopy (NIRS) sensors measure muscle oxygen saturation (SmO_2) as a performance factor in endurance athletes. The objective of this study is to delimit metabolic thresholds relative to maximal metabolic steady state (MMSS) using SmO_2 in cyclists.

Methods Forty-eight cyclists performed a graded incremental test (GTX) (100 W-warm-up followed by 30 W min) until exhaustion. SmO_2 was measured with a portable NIRS placed on the vastus lateralis. Subjects were classified by VO_2 max levels with a scale from 2 to 5: L2 = 45–54.9, L3 = 55–64.9, L4 = 65–71, L5 = > 71, which represent recreationally trained, trained, well-trained, and professional, respectively. Then, metabolic thresholds were determined: Fatmax zone, functional threshold power (FTP), respiratory compensation point (RCP), and maximal aerobic power (MAP). In addition, power output%, heart rate%, VO_2 %, carbohydrate and fat consumption to cutoff SmO_2 point relative to MMSS were obtained.

Results A greater SmO_2 decrease was found in cyclists with > 55 VO_2 max (L3, L4 and L5) vs. cyclists (L2) in the MMSS. Likewise, after passing FTP and RCP, performance is dependent on better muscle oxygen extraction. Furthermore, the MMSS was defined at 27% SmO_2 , where a non-steady state begins during exercise in trained cyclists.

Conclusion A new indicator has been provided for trained cyclists, < 27% SmO_2 as a cut-off to define the MMSS Zone. This is the intensity for which the athlete can sustain 1 h of exercise under quasi-steady state conditions without fatiguing.

Keywords Near-infrared spectroscopy (NIRS) · Muscle oxygen saturation · Exercise physiology · Metabolism and performance

Introduction

Currently, sports scientists and coaches use various parameters to classify performance in endurance athletes such as cycling and triathlon; among these, we find VO_2 max, power and heart rate at different exercise intensities [1, 2]. During

a graded exercise test (GXT) it is possible to obtain points and/or metabolic thresholds such as the fatmax zone, ventilatory thresholds, maximum aerobic power (MAP) and the functional threshold power (FTP) [3, 4], which can be used in well-trained cyclists as a practical and non-invasive alternative to estimate the maximum lactate steady state (MLSS) [5]. Also, a key parameter is the respiratory compensation point (RCP), which allows distinguishing between fatigued and non-fatigued work, this occurs due to the worsening of metabolic acidosis produced by respiratory compensation and is identified by an increase in minute ventilation (VEQ) in relation to CO_2 production (VCO_2); this generally occurs at approximately 80% VO_2 max [6, 7]. The FTP and RCP show a similar physiological mechanism due to work under fatigue conditions [8, 9], which is related to the “maximum metabolic steady state” (MMSS), representing the highest metabolic rate at which exercise can be maintained almost exclusively by oxidative metabolism, representing the upper limit of sustainable exercise [10]. Therefore, MMSS is a

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valuable component when prescribing personalised exercise. Measuring metabolic thresholds is routine in cyclists and triathletes because they can accurately track the subtle improvements in endurance performance that elite athletes attain during a season.

Heart rate and power output are often used as indicators of workload. These parameters are of interest because of their relationship with oxygen consumption during training, as wearable watches and sensors are based on these parameters to calculate the energy expenditure [11, 12]. However, there are some problems in considering HR as an indicator of internal workload, because it shows variability in unstable conditions, for example in the transition from fatigue-recovery states and psychological disorders such as stress [13]. Interestingly, in recent years portable sensors have been invented that measure muscle oxygen saturation (SmO_2), which is a variable that can be used to monitor training on a scale of 0–100% and is validated by brands such as portable MOXY [14], as used in cyclists and triathletes. From a scientific point of view, decreased SmO_2 is an indicator of better performance during cycling [15]. It also has a correlation with VO_2 , ventilatory thresholds and MLSS [16–18]. However, although there are studies that use SmO_2 during GXT to estimate ventilatory threshold points [17, 18] and training zones (moderate, heavy and severe intensity) [19], there are scientific gaps regarding the physiological behaviour during SmO_2 transitions in fatmax zone, FTP, RCP and MAP. SmO_2 during fatmax, FTP and CPR relative to MMSS could differentiate the performance level of athletes, since, as a general rule, performance is a mediator of daily training planning [20]. As such, there has been no response with a solid scientific basis, so it is important to reach an agreement and suggest a new proposal for the SmO_2 interpretation and its applicability as an internal load.

Therefore, the objective of this study was to delimit the MMSS by VO_{2max} levels using SmO_2 in trained cyclists; the following hypotheses are considered: (i) the use of SmO_2 during exercise can identify the performance level and metabolic thresholds of the cyclists, and (ii) SmO_2 can be used to determine the approximate MMSS from the FTP and RCP breakpoints. The objective of this study was to delimit the MMSS by VO_{2max} levels using SmO_2 in trained cyclists.

Methods

Participants

The sample included was 48 trained cyclists and triathletes (Experience in endurance training 10.9 ± 4.9 years and Skinfold of the vastus lateralis (ATT) 10.7 ± 5.9 mm), performance was classified by performance levels using the VO_{2max} (ml/kg/min) with a scale from 1 to 5: level

1: < 45; level 2: 45–54.9; level 3: 55–64.9 level 4: 65–71; level 5: > 71, representing untrained, recreationally trained, trained, well-trained, and professional subject groups, respectively [2]. For this study, level 1 is ruled out because the participants are athletes and no value < 45 of VO_{2max} was found. The descriptive values based on the physiological parameters of the subjects are shown in the Table 1. No physical limitations or musculoskeletal injuries that could affect training were reported. This study was conducted during the agonistic season. The study was carried out in accordance with the Helsinki Declaration and approved by the Bio-Ethical Committee of the University of Extremadura with N° registration code: 131/2018. A signed consent was obtained from each subject prior to their participation.

Experimental design

The trial design was cross-sectional. Participants carried out the test under similar environmental conditions (21–24 °C and 45–55% relative humidity) and were asked to abstain from performing intense exercise 48 h prior to the test. Before the GXT, body mass and Skinfold of Vastus lateralis values were ascertained. Then, a GXT was performed to obtain the physiological together with the SmO_2 .

Performance assessments

Maximal graded exercise test (GXT)

First, a standardized warm-up of 10 min at 100 W was performed, the set up consisted in increments 30 W·min⁻³ until exhaustion [21]. The end of the test was considered when the participant was unable to maintain the power output of each final completed stage. During GXT participants were monitored through a gas exchange measurement system/device with breath-by-breath technology and calibrated before each test (Metalyzer 3b, CORTEX Biophysik GmbH, Leipzig, Germany). Each participant used their own bike mounted on a smart training device (Bkool, model Bkool one; Madrid, Spain). The protocol was completed with a PowerTap P1 (PP1), which produced reliable output power readings of 100–500 W, in a seated position ($\rho \geq 0.987$), and an absolute reliability index (150–500 W; COV = 2.3%; SEM < 1.0 W) [22]. The PowerTap during cardiopulmonary tests are more ecologically valid, allowing cyclists to use their own bicycles [22]. HR was collected via a HR monitor (HRM-Tri; Garmin Ltd., Olathe, KS, USA). The smart trainer assessed power with internal sensors that were paired to a smartwatch for future analysis (Forerunner 735xt, Garmin, Olathe, KS, USA). Finally, when GXT was finished, a drop of capillary blood was drawn for lactate measurement (Lactate Pro 2, Arkray Factory, Inc., Amstelveen, The Netherlands), after 3 min of passive recovery [23].

Table 1 General physiological parameters based on the VO₂max levels classification obtained during the Graded Exercise test

| Performance by VO ₂ max (ml/kg/min) | N | Weight (Kg) | Age | VO ₂ max (ml/kg/min) | Lactate (mmol) | Heart Rate Maximum (ppm) | Resting Heart Rate (ppm) | Power (Watts) | Fat percentage (%) | SmO ₂ (%) Mean |
|--|----|--------------------|-----------------|---------------------------------|--------------------|--------------------------|--------------------------|--------------------|--------------------|---------------------------|
| <45 | — | — | — | — | — | — | — | — | — | — |
| 45–54.9 | 9 | 80.3 ± 6.0 (78–82) | 35 ± 5 (33–36) | 52 ± 2 (51–53) | 13.1 ± 1.7 (12–14) | 178 ± 4 (177–180) | 51 ± 8 (48–55) | 313 ± 41 (229–327) | 13.2 ± 3.0 (12–14) | 48 ± 23 (40–56) |
| 55–64.9 | 16 | 75.4 ± 7.9 (74–76) | 38 ± 5 (38–39) | 60 ± 2 (59–61) | 11.5 ± 2.2 (11–12) | 179 ± 12 (177–181) | 46 ± 7 (45–57) | 357 ± 47 (350–365) | 8.8 ± 2.2 (8–9) | 38 ± 17 (36–41) |
| 65–71 | 11 | 71.5 ± 7.8 (68–73) | 27 ± 10 (24–29) | 68 ± 2 (67–68) | 11.4 ± 2.2 (11–12) | 192 ± 10 (189–195) | 45 ± 5 (45–47) | 369 ± 59 (354–382) | 10.3 ± 1.2 (10–11) | 36 ± 21 (30–42) |
| > 71 | 12 | 71.9 ± 5.3 (71–73) | 29 ± 6 (28–31) | 75 ± 3 (74–75) | 11.0 ± 2.4 (10–11) | 185 ± 12 (183–188) | 42 ± 4 (41–43) | 388 ± 29 (382–394) | 8.8 ± 1.6 (8–9) | 38 ± 19 (37–41) |
| Pos-hoc | | a, b, c, d, e, f | a, b, d, e, f | a, b, c, d, e, f | a, d, e | a, b, e, f | a, b, c, e, f | a, b, c, e, f | a, b, d, e, f | a, d, e |

Results are presented as mean ± SD

Significantly different between levels (*p* < 0.05), pos hoc analysis = (a) 2 vs. 5; (b) 3 vs. 5; (c) 4 vs. 5; (d) 2 vs. 3; (e) 2 vs. 4; (f) 3 vs. 4

Muscle oxygen saturation assessment

Local muscle oxygen saturation (SmO₂) was assessed with a near-infrared spectroscopy (NIRS) device (MOXY, Fortiori Design LLC, Minneapolis, Minnesota, USA). Which is valid for measuring SmO₂ (ICC: *r* = 0.773–0.992) [24]. It was attached firmly to the belly of the right vastus lateralis muscle (midway between lateral epicondyle and greater trochanter of femur) using a dark elastic strap to avoid light contamination and movement artifacts. The vastus lateralis was selected based on previous evidence and considering the role of this muscle in cycling [25]. The skinfold thickness at the NIRS measurement site (VL) was measured using a skinfold caliper (Harpenden Ltd.) to ensure that the skinfold thickness was < 1/2 of the distance between the emitter and the detector. (25 mm). The raw muscle O₂ saturation (SmO₂) signal was treated with a soft spline filter to reduce noise created by movement [26] using a Minitab 19 (Minitab, Inc, State College, PA; www.minitab.com, USA).

For the data analysis the following guidelines were followed:

- (1) The average value of the last minute of the 3-min step was used.
- (2) Data was excluded when changes in SmO₂ > 10% were observed, compared to the previous SmO₂ value, this was considered as measurement error.
- (3) Data that gave 0% of reading were excluded due to the apparent lost signal. The data was viewable in real time to the NIRS technology expert researchers and muscle oxygenation measurement, using ANT + technology software (Golden Cheetah version 3.4, USA) and joint data processing software (Excel 2016, Microsoft Office 365, USA).

Gas exchange analysis to determine metabolic thresholds

Fatmax Zone: The Fat oxidation (FAT), carbohydrate oxidation (CHO) were calculated using appropriate stoichiometric equations [27] and energy equivalence, based on the measured values achieved

$$FAT \left(\frac{kcal}{min} \right) = (1.67 \times VO_2) - (1.67 \times VCO_2) * 9,$$

$$CHO \left(\frac{kcal}{min} \right) = (4.55 \times VCO_2) - (3.21 \times VO_2) * 4.$$

Data analysis to determine Fatmax involved the measured values approach in the stage with the highest recorded fat oxidation value and the corresponding VO₂ [21].

Maximum Metabolic Steady State (MMSS): MMSS is considered to be the highest oxidative metabolic rate that can be sustained during continuous exercise. However, there are several indices that are often used to identify the MMSS [28]

1. Functional Threshold Power (FTP): The FTP was calculated with the maximum incremental cycle test methodology until exhaustion to find the resulting maximum power, from which the FTP is obtained by the following equation [29]:

$$\text{FTP} = \text{Outputpower}(W) \times 0.865 - 56.484.$$

2. Respiratory Compensation Point (RCP): The RCP is defined as the abrupt and positive “acceleration” in VE/VO_2 versus VO_2 and VE/VCO_2 versus VO_2 data [30], and was confirmed by a simultaneous reduction of the end-expiratory pressure of CO_2 [20] This was determined by 2 independent exercise physiologists by visual inspection. We also provide the VEQ values as a reference for each performance group. V-slope load was identified in that intensity of exercise which, in a plot of the minute production of CO_2 over the minute utilization of oxygen (VO_2), shows an increase in the slope above 1.0 [31]. The VO_2max was defined as the highest plateau (two successive maximal within $150 \text{ mL}\cdot\text{min}^{-1}$, averaging the data every 5 s) reached [32].
3. SmO_2 Transition to metabolic threshold: The transition point of muscle oxygenation was identified as the difference in the SmO_2 values from FTP to RCP/VT2.

Statistic analysis

A descriptive analysis of the data extracted from the incremental test was applied and the Shapiro Wilk normality test was applied for each variable. When normality was reached, the two-way repeated measures analyses of variance ANOVA test was performed to identify the effects of

the two categories: VO_2max levels and metabolic thresholds relative to MMSS. Then a bonferroni post hoc was applied to identify the internal differences between the groups. In addition, the receiver operating characteristic (ROC) curves was used to establish a cutoff for SmO_2 at the MMSS value, and the area under curve (AUC) was used to evaluate the performance of a classifier, where the threshold cut-off values were defined by the points representing the highest concomitant sensitivity and specificity, the AUC was interpreted according to with the following guidelines: not informative ($\text{AUC} = 0.5$), less accurate (0.5–0.7), moderately accurate (0.7–0.9), high precision (0.9–1) and perfect discriminatory test ($\text{AUC} = 1.0$) [33]. Also, a differential analysis of the performance variables was performed using the SmO_2 cut-off established in relation to MMSS. The level of significance was established at $p < 0.05$ with 95% confidence intervals. The results were expressed as mean \pm standard deviation. All analyzes were performed with SPSS software (version 22).

Results

Table 1 shows the mean values of the general physiological parameters during a GET, differences were found in level 2 vs level 3, 4 and 5, in the variables weight, VO_2max ($p \leq 0.001$), HRmax ($p \leq 0.001$), Resting HR ($p \leq 0.001$), Lactate ($p \leq 0.001$), Power W ($p \leq 0.001$), Fat% ($p = 0.000$) and SmO_2 ($p \leq 0.005$), observing a worse performance at level 2.

Table 2 shows the SmO_2 values comparing by performance levels in the metabolic thresholds: Fatmax, transition between FTP and RCP and MAP. Differences were found in level 2 vs level 5 ($\text{FO } \Delta = 17 p \leq 0.005$; $\text{FTP } \Delta = 14 p \leq 0.001$; $\text{RCP } \Delta = 17 p \leq 0.001$; $\text{MAP } \Delta = 12 p \leq 0.001$). Likewise, differences were found between level 2 vs level 3 and 4 in SmO_2 at the FTP and RCP (SmO_2 difference of

Table 2 Comparison of muscle oxygen saturation (%) by VO_2max levels during metabolic thresholds in cyclists and triathletes

| Levels | VO_2max (ml/kg/min) | SmO_2 Fatmax | Maximal Metabolic Steady State | | | SmO_2 Maximum Aerobic Power (MAP) |
|--------|--|--------------------------|---|---|---|---|
| | | | SmO_2 breaking point to FTP | SmO_2 Transition to metabolic threshold | SmO_2 breaking point to RCP/VT2 | |
| 1L | < 45 | – | – | – | – | – |
| 2L | 45–54.9 | 59 ± 21 (30–93) | 42 ± 14 (34–45) | 2 ± 0.1 | 40 ± 15 (34–42) | 22 ± 14 (10–40) |
| 3L | 55–64.9 | 53 ± 10 (36–70) | 31 ± 8 (28–33) | 5 ± 0.5 | 26 ± 8 (25–28) | 19 ± 6 (15–22) |
| 4L | 65–71 | 48 ± 14 (42–54) | 30 ± 14 (26–32) | 6 ± 0.7 | 24 ± 10 (23–25) | 10 ± 2 (7–14) |
| 5L | > 71 | 42 ± 8 (35–50) | 28 ± 10 (26–28) | 5 ± 0.4 | 23 ± 4 (20–25) | 12 ± 3 (10–15) |
| | Pos-hoc | a | a, d, e | | | a, e |

Results are presented as mean \pm SD

*Significantly different between levels ($p < 0.05$), pos hoc analysis = (a) 2 vs. 5; (b) 3 vs. 5; (c) 4 vs. 5; (d) 2 vs. 3; (e) 2 vs. 4; (f) 3 vs. 4

levels 2 vs levels 3 and 4 $\Delta = 18$; $\Delta = 21$; $\Delta = 22$; $p \leq 0.001$ and $\Delta = 13$; $\Delta = 16$, respectively).

Figure 1 represents the ROC curve to assess the cut-off of SmO₂ and maximum metabolic steady state in relation to FTP and CPR, established from VO₂max level 3 (>55 ml/kg/min). We found an 63% (AUC) with a SE = 0.039; $p = 0.001$ and lower and upper limits = (0.55–0.70%), which indicates a value of 26.80 for SmO₂, Sensitivity = 0.595 and Specificity = 0.558, hence we propose 27% SmO₂ as an approximation to MMSS and <27 SmO₂ as a reference for the fatigue thresholds.

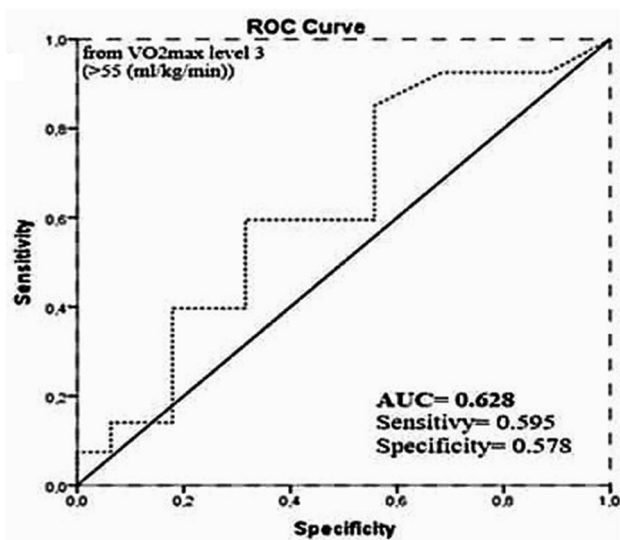


Fig. 1 An analysis of the receptor operating characteristics (ROC) curve to evaluate the cut-off for muscle oxygen saturation and maximum metabolic steady state

Fig. 2 Reference of muscle oxygen saturation at the maximal metabolic steady state: comparison by VO₂max levels

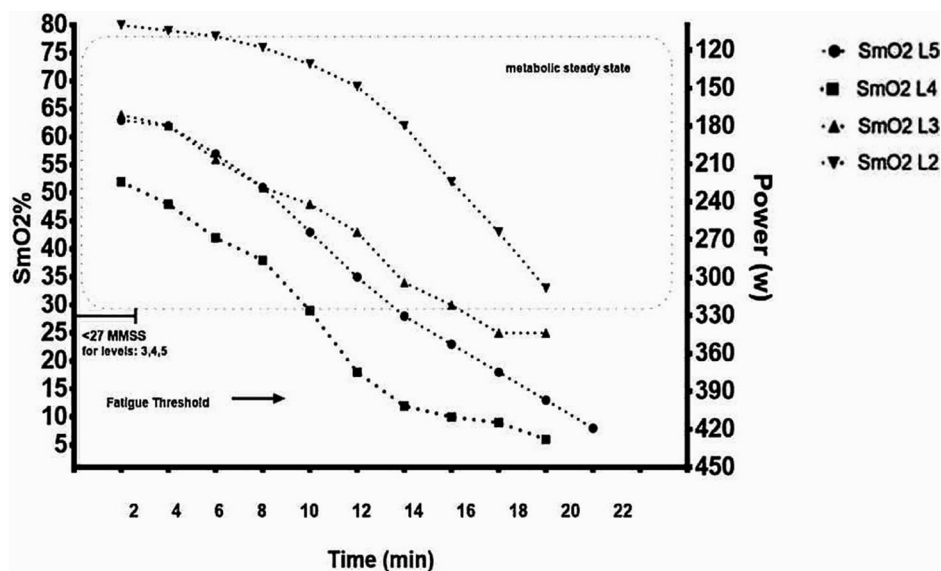


Figure 2 shows differences by VO₂max levels with the SmO₂ kinetics during GET. First, we observed better MSSS and better SmO₂ performance at levels 4 and 5 (W:289 ± 51; SmO₂: 26 ± 8 and L5 = W: 301 ± 25; SmO₂: 24 ± 4) vs 2 and 3 (L2 = W: 214 ± 35; SmO₂: 40 ± 14 and L3 = W: 252 ± 41; SmO₂: 29 ± 8 $p \leq 0.001$). It was also observed that levels 3, 4 and 5 use less SmO₂ than level 2 after passing MMSS (L:3 9 ± 6; L4:10 ± 7 and L5: 7 ± 8 vs 15 ± 8 $p \leq 0.001$).

Likewise, we highlight that before exceeding the MMSS, the use of oxygen is similar in all the performance groups where no difference was observed (L2: 26 ± 15; L3: 28 ± 17; L4: 27 ± 19 and L5: 22 ± 18, respectively). Finally, it can be seen that in levels 3, 4 and 5 the SmO₂ vs the output power is reached close to 27%, in level 2 a delay is observed and SmO₂ at the MMSS is greater than 27% (possibly between 40 and 45%).

Table 3 shows the differences by VO₂max levels, using the reference point of 27% SmO₂, considering it as a fatigue threshold, once the MMSS is exceeded. Also, shows physiological parameters. Differences were found between level 2 y 3 with the level 5 in time (L5 vs. L2: $\Delta = 4.0$ $p \leq 0.005$; L3: $\Delta = 2.0$ $p \leq 0.001$) and W/kg (level 5 vs level 2: $\Delta = 0.71$ $p \leq 0.005$; level 3: $\Delta = 0.44$ $p \leq 0.005$), also is observed that the level 5 group consumed more fat when reaching the 30% SmO₂ point (L5 vs. L2: $\Delta = 2.1$ $p \leq 0.001$; L3: $\Delta = 1.9$ $p \leq 0.001$ $\Delta = 1.7$ $p \leq 0.001$).

Discussion

This study used SmO₂ to check performance differences by VO₂max levels through the measurement of metabolic thresholds associated with MMSS such as FTP and RCP. The main finding is that subjects trained from a VO₂max > 55 can

Table 3 Comparison of physiological parameters at “27% SmO₂” by performance levels in cyclists and triathletes

| Levels | VO ₂ max (ml/kg/min) | Time (min) | VO ₂ max (%) | Heart Rate (%) | Power (W%) | Carbohydrates (Kcal/min) | Fat (Kcal/min) | W/Kg |
|-----------|---------------------------------|------------|-------------------------|----------------|------------|--------------------------|----------------|---------|
| 1L | <45 | – | – | – | – | – | – | – |
| 2L | 45–54.9 | 13±4 | 80±14 | 87±13 | 89±18 | 10.8±3.3 | 2.4±2.9 | 3.2±1.0 |
| 3L | 55–64.9 | 14±3 | 75±12 | 87±8 | 80±24 | 12.6±2.5 | 2.2±1.9 | 3.3±0.7 |
| 4L | 65–71 | 16±3 | 67±10 | 85±3 | 72±12 | 11.7±3.8 | 3.2±3.0 | 3.5±0.8 |
| 5L | >71 | 18±7 | 66±8 | 82±7 | 72±11 | 11.4±4.6 | 6.1±2.9 | 3.9±0.7 |
| | Pos-hoc | a, b | | | | | a, b, c | a, b |

Results are presented as mean ± SD

*Significantly different between levels ($p < 0.05$) = (a) 2 vs. 5; (b) 3 vs. 5; (c) 4 vs. 5; (d) 2 vs. 3; (e) 2 vs. 4; (f) 3 vs. 4

take into consideration SmO₂ values of <27% as the internal training load nears FTP and RCP. Also, it is assumed that below 27% SmO₂ the maximum metabolic steady state occurs and is observable by critical oxygenation, as recently discovered by Feldmann and Erlacher [34].

According to the findings, athletes with a greater training status (higher VO₂max levels) achieved a better performance in reducing SmO₂ values [15]. A greater SmO₂ decrease is related to power output increase [24, 34]. Likewise, our study determined that SmO₂ in the fatmax zone only showed a difference with athletes of Level 5 “>71 VO₂max” vs. Level 2 “<55 VO₂max”; however, in the MAP zone there is only a difference between the greater capacity “>65 VO₂max”. This explains that the differences between athletes in energy expenditure during high-intensity exercise and the use of muscle oxygen to generate ATP as metabolic fuel [35]. Figure 2 also shows a greater SmO₂ decrease in athletes with higher VO₂max after passing the FTP and CPR breakpoint. Therefore, fatigue resistance depends on the ability to maintain critical oxygenation (CO) over time with lower SmO₂ values [19]. OC is the ability of the muscle to maintain adequate oxygen supply to match oxygen demand as the basis for steady-state exercise theory [34, 36]

In our study, athletes >65VO₂max achieved greater desaturation levels than less highly trained athletes; this is supported by Fontana et al. [37], who found that the oxygen used after passing the RCP is lower and depends on the capacity for ATP production that comes from non-oxidative metabolic pathway. To explain the physiological mechanisms of the progressive decrease in SmO₂ and increase in VO₂ from a systemic perspective, it is the result of a linear increase in cardiac output (i.e., systemic blood flow) and a hyperbolic increase in the arterio-venous O₂ difference (that is, systemic O₂ extraction) until depletion [38]. Portable NIRS-derived local O₂ extraction (SmO₂) within active tissues does not show the same hyperbolic profile as systemic measurements, indicating that the central and peripheral profiles of O₂ extraction and blood flow are different [39]. However, these studies did not discuss the changes

in metabolic pathways within the muscle, which, once the MMSS-related with fatigue thresholds are exceeded, are less dependent on oxygen to generate power [40–42]. As a result, there is a muscle O₂ extraction reserve that can only be used at the GTX end, that is, at high intensities (>85% VO₂max), which is observable in a greater SmO₂ decrease and greater oxygen extraction capacity by the muscle [43]. This was seen in our study, as higher VO₂max can desaturate more oxygen, so SmO₂ can be considered as a performance factor in high intensity exercise.

Likewise, a better interaction between the supply and extraction of O₂ by the muscle depends on the recruitment of motor units covered by the muscle fibres; in this sense, the type II fibres are activated to a higher level after the FTP and RCP breakpoints, and, therefore, there is a metabolic pathway change; being an ‘oxygen independent’ energy system [44], a lower extraction of muscle oxygen was observed since type II fibres have less oxidative capacity and need less oxygen to function, but they achieve better performance in the high-intensity zones due to their glycolytic capacity to maintain a greater force and power production [42, 43]. This, has also been demonstrated in the greater progressive recruitment of fast-contracting motor units (type II fibres) in the vastus lateralis muscle with EMG at the end of the exercise (>80%) where there is less extraction of tissue oxygen [45].

On the other hand, among the factors that explain the SmO₂ decrease after FTP and RCP are an increase in the oxygen partial pressure and hydrogen (H), a decrease in pH and the CO₂ partial pressure within the active muscles [46], which cause a vasodilator response when the high intensity exercise cannot be sustained [47]. The demand of oxygen extraction then follows in a hyperbolic and non-linear way, due to the muscles’ attempts to restore PCr/ATP by the non-oxidative pathway, which is why small increases in muscle oxygen occur during the heavy and severe intensity domains (VO₂ phase II and III) [43]. Likewise, previous studies determined that the VO₂ slowing at the exercise end causes an increase in the respiratory

muscle work, and this attenuates the increase in blood flow and O_2 transport in the legs [48]. This also supports the proposal to use $< 27\%$ SmO_2 in trained subjects, not as a breakpoint but rather as a fatigue threshold. Therefore, it is necessary to understand that critical oxygenation occurs after this threshold $< 27\%$ SmO_2 .

Finally, we highlight that there is already a breakpoint in oxygenated HBO within the muscle; that is, in a similar way to SmO_2 , the signal from this breakpoint is a reliable biomarker of exercise intensity which is closely associated with VO_2 at the RCP [49, 50], since it occurs at the same metabolic rate [37]. The RCP is close to 27% SmO_2 (between 26 and 30%) observed in AUC (Fig. 1), which indicates that the MMSS may be before or after 27% SmO_2 , depending specifically on the degree of training. In this sense, Table 3 shows that when comparing all of the performance variables at the established point from 27% of each subject, no differences were found. This means that a gold-standard point within the SmO_2 scale (0–100%) demarcates the intensity domain; because it will always be the same value. For example, with improvements in the $VO_{2max}\%$ level, the fatigue zone would be delayed and better tolerated. Therefore, we suggest that the SmO_2 value relative to MMSS represents the same metabolic rate for all groups. Our study found differences only in fat consumption (kcal/min) and with the greater capacity to produce power per weight (W/kg) in Level 5 vs Level 2 because the oxidative capacity resulting from the greater use of oxygen by type II fibres is inversely correlated with exercise economy and a lower capacity to produce power output [51]. It was observed that those at Level 5 maintained more power with the use of lower $VO_{2max}\%$ (Table 3). This approach should be tested with different training programs and GXT protocols.

Limitations and recommendations

A limitation of our study was the non-measurement of critical power (CP), which denotes the transition from the intensity of heavy-severe exercise with relatively little error (SE, 11%), while the MMSS tend to underestimate by 11%. Also, RCP and VT2 overestimate CP by 6 and 21%, respectively [52]; therefore, 27% SmO_2 is not an exact point and may overestimate some untrained athletes and non-cyclists. Also, during training, the cadence must be taken into account, since the use of muscle oxygenation is affected by acceleration [53]. Although there were small differences in the use of CH, HR%, power% and $VO_{2max}\%$ in our study (Table 3), they were not statistically significant. This does not mean that it is not possible to train for targets over fatigue threshold or set point of $< 27\%$ SmO_2 , but this must be tested with seasonal training effects.

Conclusion

To summarise, trained cyclists and triathletes with $a > 55$ VO_{2max} (ml/kg/min) could use values lower than 27% SmO_2 as high-intensity zones and breakpoints relative to FTP and RCP, which is the intensity at which the athlete can sustain 1 h of exercise in quasi-steady-state conditions without fatiguing. Also, a greater SmO_2 decrease is better in more highly trained athletes, this can be used to measure peripheral adaptations to training.

Author contributions GO and AG contributed to the conception of the study. AV, RT and GO designed and reviewed the protocol of the study. AG performed the data collection. AV and AG organised the database. AV and AG performed the statistical analysis. AV and RT wrote the first draft of the manuscript. AV, RT and GO wrote sections of the manuscript. RT and GO supervised the project. All authors contributed to manuscript revision, read, and approved the submitted version.

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Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval The study was carried out in accordance with the Helsinki Declaration and approved by the Bio-Ethical Committee of the University of Extremadura with N° registration code: 131/2018.

Informed consent A signed consent was obtained from each subject prior to their participation.

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