A comparative analysis of mathematical methods for detecting lactate thresholds using muscle oxygenation data during a graded cycling test.

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Threshold determination for improving training and sports performance is important for researchers and trainers, who currently use different methods for determining lactate, ventilatory or muscle oxygenation (SmO_2) thresholds. Our study aimed to compare the identification of the intensity at the first and second thresholds using lactate and SmO_2 data by different mathematical methods in different muscles during a graded cycling test. Twenty-six cyclists (15 males and 11 females; 23 ±6 years, 1.71 ±0.09 m, 64.3 ±8.8 Kg and 12 ±3 training hours per week) performed a graded test on the cycle ergometer. Power output and saturation of muscle oxygen in four muscles (vastus lateralis, biceps femoris, gastrocnemius and tibialis anterior) were measured, along with systemic lactate concentration. Our results showed that any method was reliable for determining the first muscle oxygenation threshold (MOT1) when comparing the lactate threshold in any muscle. However, the best method for determining the second muscle oxygenation threshold (MOT2) was the Exp-Dmax ($p < 0.01$; ICC=0.79-0.91) in all muscles. In particular, the vastus lateralis muscle showed the highest intraclass correlation coefficient (ICC=0.91, CI95%[0.81, 0.96]). However, results varied per sex across all muscles analyzed. In conclusion, although the first muscle oxygenation threshold could not be determined using mathematical methods in all the muscles analyzed, the Exp-Dmax method presented excellent results in detecting the second systemic threshold in the vastus lateralis.

Keywords: breakpoint; aerobic; anaerobic; cyclists; vastus lateralis; incremental test.

1. Introduction

Since the 1970s, various studies have discussed the importance of threshold determination for improving training and sports performance (Mann et al., 2013). Threshold is defined as the magnitude or intensity that must be exceeded for a certain reaction, phenomenon, result or condition to be manifested (Poole et al., 2021). Currently, different methodologies for determining thresholds at the systemic level, such as lactate and gas exchange, are commonly used (Caen et al., 2021). In contemporary physiology, lactate is a biomarker of physiological and metabolic strain, this is produced under fully aerobic conditions (Brooks et al., 2022). Then, the lactate threshold represents the inflection point when the body begins to produce lactate at a faster rate than it can eliminate (Poole et al., 2021). However, sports scientists are seeking greater control of training loads on a localized basis (Seshadri et al., 2019), and technologies for field acquisition, such as muscle oximetry using near-infrared spectroscopy (NIRS), have been improved for this purpose (Perrey & Ferrari, 2018).

NIRS is a non-invasive technology and in some cases portable technology, which has been used in different sports (Perrey & Ferrari, 2018), from team sports such as rugby (Jones et al., 2013) to endurance sports such as testing athletes in cycling or running (Feldmann et al., 2022). This technology detects the heme compound of hemoglobin, myoglobin and cytochrome oxidase, so as to calculate muscle oxygen saturation (SmO_2) using the main absorbing chromophores found in skeletal muscle (Barstow, 2019). Hence, when light detects hemoglobin and myoglobin with an iron core associated with the heme component, its light absorption varies (Barstow, 2019). For this reason, SmO_2 depends on this relationship, but the device does not differentiate between the components (hemoglobin and myoglobin) (Feldmann et al., 2019). The NIRS oximeters use a scale from 0 to 100 % for continuously measuring the hemoglobin and myoglobin charged with oxygen (Perrey & Ferrari, 2018). This technology has the drawback that high skinfolds in the location of the device could affect SmO_2 measurement (Gandia-Soriano et al., 2022; Niemeijer et al., 2017).

SmO_2 data has been suggested as a reliable for detecting thresholds because it shows a continuous decline during graded cycling test (GXT) with a change in slope near the second lactate threshold (Cayot et al., 2021; Hovorka et al., 2023; Salas-Montoro et al., 2022; van der Zwaard et al., 2016). However, there is a lack of knowledge about the best mathematical method for determining the first and second SmO_2 thresholds (MOT1 and

MOT2, respectively). One example of the diversity in identifying this is that while some studies determined the threshold by calculating a nonlinear increase point (Spencer et al., 2012; Yogev et al., 2022), others identified the breakpoint of the SmO_2 visually (Rodrigo-Carranza et al., 2021; Salas-Montoro et al., 2022). Moreover, Cayot et al. (2021) used mathematical methods (Dmax and mDmax methods) to determine the lactate and MOT thresholds. However, most of these studies only used one portable muscle oxygen monitor (Driller et al., 2016; Raleigh et al., 2018) and few investigations have used more than one (Beever et al., 2020; Contreras-Briceño et al., 2019), which makes it interesting to explore how the thresholds detected is more related with the systemic lactate threshold depending on the muscle function (e.g., power-generating muscles vs stabilizer muscles) in the exercise performed.

There are different mathematical methods that are used for both lactate and oxygen consumption to automatically detect linearity changes (Jamnick et al., 2020). These methods are available to trainers and athletes through free software and for this reason is interesting to test whether such methods can be used with SmO_2 data. In addition, considering that previous research has observed that the MOT2 in the muscle most involved in the exercise occurs at a similar moment to the second threshold of lactate (Cayot et al., 2021; Salas-Montoro et al., 2022), it is interesting to evaluate these methods with lactate data as a reference. The aim of this study was to compare the identification of the intensity at the first and second thresholds using lactate and SmO_2 data by different mathematical methods in different muscles during a GXT

2. Material and methods

2.1. Participants

The study was carried out with a sample of twenty-six cyclists (15 male and 11 female) categorized as competitive (Priego Quesada et al., 2018), who were recruited from cycling clubs in response to an online questionnaire. The sample size of 26 participants was calculated based on an expected intra-class correlation coefficient (ICC) of 0.80 based on a previous meta-analysis (Sendra-Pérez et al., 2023), with a precision of ±0.1, a confidence level of 80%, two groups compared, and an expected dropout rate of the 10% (Arifin, 2023; Walter et al., 1998). Some considerations were peformed regarding the menstrual cycle state of the female participants due to the possible hormonal effect on performance (McNulty et al., 2020). The eumenorrheic females went to the laboratory during the early days of the follicular phase (Elliott-Sale et al., 2021), and the females that used a contraceptive pill during the abstinence week (Sims & Heather, 2018). The distribution of females that were included in the study was: 6 eumenorrheics, 2 ingested contraceptive pills and 3 amenorrheics or with irregular menstrual cycle. The characteristics of the participants are shown in Table 1.

Table 1. Mean and standard deviation of the characteristics of the trained cyclists assessed.

Characteristic	All participants	Male (N=15)	Female (N=11)	Male vs Female *p-value (ES)*
Age (years)	23 ±6	23 ±7	22 ±4	0.9
Height (m)	1.71 ±0.09	1.78 ±0.05	1.64 ±0.04	**<0.01 (2.8)**
Body mass (kg)	64.3 ±8.8	70.2 ±5.3	58.3 ±8.1	**<0.01 (1.7)**
Body mass index (kg/m²)	21.96 ±2.08	22.1 ±1.55	21.71 ±2.72	0.2
Training hours (hours/week)	12 ±3	12 ±3	12 ±3	0.7
Experience years (years)	9 ±5	10 ±6	7 ±3	0.3
Calf skinfold (mm)	14.2 ±5.1	11.3 ±3.9	16.6 ±5.1	**0.01 (1.1)**
Thigh skinfold (mm)	20.1 ±5.8	16.7 ±4.5	22.3 ±5.9	**<0.01 (1.2)**

ES: Cohen effect size; p-value was obtained by the T-Student test

The study protocol was reviewed and approved by the Ethics Committee of the University of BLINDED (No. 2630853), and follows the ethical principles for medical research in humans of the Declaration of Helsinki. Participants were informed of the risks and benefits of participating in the study and signed the informed consent before starting the protocol.

2.2. Protocol

Participants were instructed to sleep at least seven hours the night before the test and to not consume any stimulant substance nor perform exercise 24 hours before. The protocol was performed on a cycle ergometer (Cardgirus W3, Sabadell, Spain), which was previously adjusted to the bike measurements (saddle height, saddle setback and handlebar height) that the cyclists had sent to the researcher guided by the instructions provided by the researchers. In addition, they were asked which of their leg and arm were dominant (Melick et al., 2017).

The test started with a 3-min warm-up and a constant load of 1 $W.Kg^{-1}$, and then, consisted of 3-min each step as recommended by Bentley et al. (2007), with power increments of 0.5 $W.Kg^{-1}$ during the first 10-sec (Possamai et al., 2020). Cyclists maintained a constant cadence of 90 ±10rpm during the test. The test ended when the cadence decreased below 80 rpm for 10-sec or the participant decided to give up the test.

2.3. Procedures

The normalized power output ($W.Kg^{-1}$) was measured by means of the cycle ergometer. The SmO_2 (%) was measured using NIRS technology (Moxy, Fortiori Design LLC, Minneapolis, USA) during the entire test. The Moxy calculates SmO_2 according to the amount of light reaching two detectors at 12.5 and 25 mm from the emitter (McManus et al., 2018). Four Moxy devices were placed in different locations based on surface electromyography guidelines (Hermens et al., 1999) by the same researcher in all interventions: I) vastus lateralis at 2/3 of the distance between the line from the anterior spina iliac superior to the lateral side of the patella; II) biceps femoris on the long head at 1/2 of the distance between the line between the ischial tuberosity and the lateral epicondyle of the tibia; III) gastrocnemius medialis at the most prominent bulge of the muscle and IV) tibialis anterior at 1/3 of the distance between the line between the tip of the fibula and the tip of the medial malleolus. Devices were fastened using medical adhesive tape (Hipafix, BSN Medical, Hamburg, Germany). The SmO_2 data were obtained with a frequency of 0.5 Hz. Skinfold calf and thigh were measured (mean of two measurements) by the same researcher using a plicometer (Boz-Calpro-3, Bozeera, China) following the protocol for anthropometric assessment of the International Society for the Advancement of Kinanthropometry (ISAK). Moreover, the blood lactate concentration was measured ($mmol.L^{-1}$) using Lactate Pro 2 (AKRAY Europe BV Amstelveen, Netherlands), in the second drop of blood in the ear lobe that was extracted in the last 30-sec of each step (each step had a duration of 3-min). Previous studies showed that Lactate Pro 2 presented a good reliability compared with blood samples (Crotty et al., 2021; Raa et al., 2020), with higher agreement if the measurement is performed in the earlobe than in the fingertip (Raa et al., 2020), and with Bland Altman 95% limits of agreement varied from ± 0.3 $mmol.L^{-1}$ to +1.4 $mmol.L^{-1}$ for concentrations lower than 4.0 $mmol.L^{-1}$ and higher than 8.0 $mmol.L^{-1}$, respectively (Crotty et al., 2021).

2.4. *Determining lactate and SmO_2 thresholds*

Training zones are commonly defined by determining two physiological breakpoints (e.g., VO_2, blood lactate, heart rate) through applying the three-phase model of intensity (Ribeiro et al., 2015; Stergiopoulos et al., 2021). In this study, lactate and SmO_2

breakpoints were calculated using the *"lactater"* package in RSTUDIO (version 2022.02.03).

The SmO_2 data to determine the thresholds was previously filtered using a low-pass filter (cut off frequency of 0.2) (Fig. 1.B) After that, the variation was calculated compared to the mean of the warm-up (Fig. 1.C). Finally, the data were inverted to make the SmO_2 behave incrementally instead of decrementally (Fig. 1.D). All these steps were taken to make sure that the SmO_2 data had similar behavior to the lactate and, therefore, could be processed by automatic methods currently used for lactate thresholds (Fig. 1.E).

The methods used in the study were (Fig. 1.E): (1) Log-log is a model that determines the first threshold using a linear regression method (Beaver et al., 1985). (2) Dmax is a point on the data curve at the maximum distance from the line drawn between the curve's lowest and highest values using a third-order polynomial, so determining the second threshold (Bishop et al., 1998; Driller et al., 2016). (3) Modified Dmax (ModDmax) is similar to Dmax but, the start point is different because the line is drawn between 0.4% above the lowest value and the maximum value (Bishop et al., 1998; Driller et al., 2016). (4) Log-Exp-ModDmax is a method for detecting the second threshold. Exercise intensity produces the maximum perpendicular distance to the straight line between the Log-log and the last data point of the data curve. A 3rd-order polynomial regression curve is applied (Jamnick et al., 2018). (5) Exponential Dmax (Exp-Dmax) uses an exponential curve. The breakpoint is the point that produces the maximum perpendicular distance to the straight line between the first and the last data value (Hughson et al., 1987). (6) LTP1 and LTP2 use segmented regressions. LTP1 is calculated using the first increase above baseline in data and LTP2 uses the second increase (LTP2) (Hofmann & Tschakert, 2017). (7) LTratio is a method that calculates the first threshold using the lowest value of the ratio data performance (Dickhuth et al., 1999).

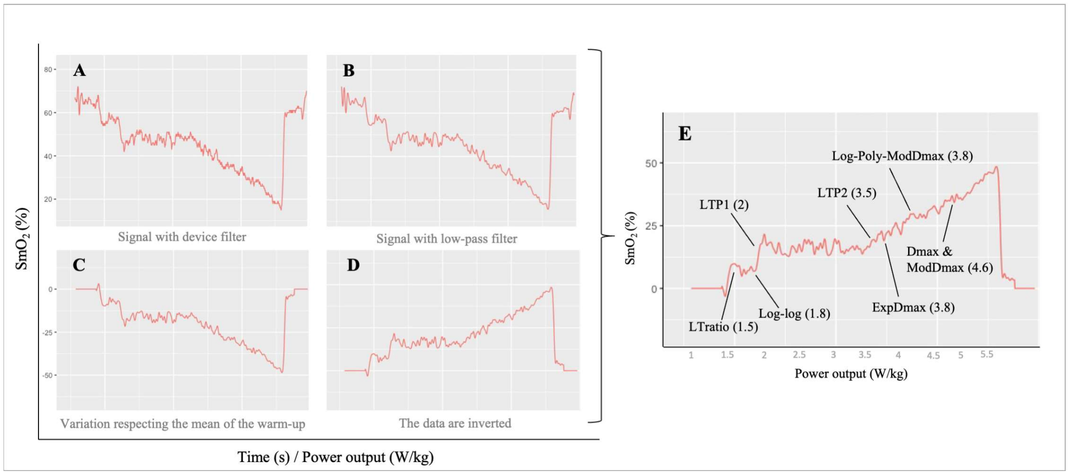

Fig. 1. Example of using muscle oxygen saturation for determining thresholds in the dominant vastus lateralis of a participant. Graphs shows the processing of the signal: (A) Signal with device filter; (B) signal with a low-pass filter; (C) variation respecting the mean of the warm-up; (D) sign data inversion; (E) the mathematical methods used for threshold determination using muscle oxygen saturation data, the values within the parenthesis being the intensity defined for each method in W.Kg^{-1}.

2.5. Statistical analysis

Statistical analyses were performed using RStudio (version 2022.02.03). The normality of data distribution was tested using Shapiro-Wilk. Initially, the discrepancies in automatically applying the different methods were calculated and represented. Later, the differences in intensity determination (W.Kg^{-1}) between lactate and SmO_2 data using the different methods were assessed using Wilcoxon tests. For significant differences ($p<0.05$), Cohen's effect sizes (ES) were computed and classified as small (ES 0.2–0.5), moderate (ES 0.5–0.8), or large (ES>0.8) (Cohen, 1988). In addition, the intraclass correlation coefficients (ICC) between lactate and SmO_2 data using the different methods were obtained, based on a single rater-measurement, absolute-agreement, and 2-way random-effects model. ICC values were classified such as: 1.00-0.81 (excellent), 0.80-0.61 (very good), 0.60-0.41 (good), 0.40-0.21 (reasonable) and 0.20-0.00 (deficient) (Weir, 2005). Lastly, for the method with the highest ICC values obtained, Bland-Altman plots (Altman & Bland, 1983) were obtained to examine whether the thresholds calculated using lactate and SmO_2 were in agreement.

3. Results

3.1. *Cases which the automatic methods not worked correctly*

The automatic methods used in some cases did not work or did not determine the threshold or the values were incoherent (i.e., less than intitial value $1W.Kg^{-1}$). For this reason, the percentage of cases that worked correctly in each method and muscle was calculated (Table 2). The muscle with the least percentage of cases where no detected the threshold was the vastus lateralis (4%), while the methods with the highest number of cases that worked incorrectly were Dmax and LTratio with 12-13%. The automatic methods not worked correctly in muscles were primarily found in females. The muscle with the most discrepancies was the gastrocnemius, with six discrepancies, four of which were in females. Additionally, the biceps femoralis had three discrepancies, 67% of which were in females.

Table 2. Percentage of cases that worked correctly in muscles and methods when thresholds were calculated.

	Vastus lateralis	**Biceps femoralis**	**Tibialis anterior**	**Gastrocnemius**	**Mean**
Log-log	96	88	96	77	89
Dmax	96	88	92	73	88
ModDmax	96	88	96	77	89
Exp-Dmax	96	88	96	77	89
Log-Exp-ModDmax	96	88	96	77	89
LTP1	96	88	96	77	89
LTP2	96	88	96	77	89
LTratio	92	88	92	73	87
Mean	**96**	**88**	**95**	**76**	**89**

3.2. Differences between the muscle oxygenation threshold and lactate threshold

Table 3 shows the descriptive data of the lactate and SmO_2 value when threshold were detected, and from these values and to be able to carry out the comparison statistics between both variables, it was calculated the power output of the GXT when each of the thresholds occurred (i.e., MOT1 and MOT2 in muscle oxygenation threshold and LT1 and LT2 in lactate thresholds)

Table 3. Mean and standard deviation of the lactate and muscle oxygen saturation (SmO_2, variation respecting the mean of the warm-up with the sign inverted) in which tresholds were calculated for each muscles and method. T1: first threshold. T2: second threshold .

	Lactate (mmol.L^{-1})	**Vastus lateralis**	**Biceps femoralis**	**Tibialis anterior**	**Gastrocnemius**
		SmO_2 (%)	SmO_2 (%)	SmO_2 (%)	SmO_2 (%)
LTP1	1.7 ±0.4	12 ±12	-3 ±9	7 ±10	-1 ±12
LTP2	5.0 ±1.8	32 ±17	6 ±17	15 ±13	12 ±14
Dmax	2.8 ±0.8	31 ±18	3 ±15	13 ±13	12 ±17
ModDmax	3.8 ±0.8	32 ±17	5 ±15	13 ±13	7 ±13
Exp-Dmax	3.3 ±0.9	24 ±12	5 ±9	11 ±9	5 ±9
Log-Exp-ModDmax	4.5 ±1.1	34 ±16	15 ±14	16 ±10	14 ±13
Log-log	1,7 ±0.4	24 ±15	7 ±9	9 ±8	10 ±7
LTratio	1.8 ±0.4	2 ±10	-6 ±6	1 ±7	-5 ±7
Mean T1	1.7 ±0.4	12 ±12	-1 ±8	6 ±9	1 ±9
Mean T2	3.9 ±1.1	30 ±16	7 ±14	14 ±12	15 ±14

Figures 2, 3 and 4 show the determination of the muscle threshold using SmO_2 and lactate in all the participants, males and females, respectively. In general, SmO_2 threshold presented higher values than the lactate in all muscles for Dmax (except biceps femoris in males), LTP1 (except vastus lateralis and gastrocnemius in males), Log-Exp-ModDmax ($p<0.05$ and $ES>0.6$), and Log-log method (except in tibialis anterior for males) ($p<0.05$ and $ES>0.6$). LTratio was higher for lactate than SmO_2 ($p<0.05$ and $ES>0.5$), except in the females, where no differences were observed. Finally, the methods without differences in most cases of determining the threshold between SmO_2 and lactate were ModDmax (except for vastus lateralis, biceps femoris and tibialis anterior of the females and gastrocnemius of all participants), Exp-Dmax (except for biceps femoris of males and tibialis anterior of the females) and LTP2 (except for gastrocnemius of all participants, and vastus lateralis, gastrocnemius and tibialis anterior of females).

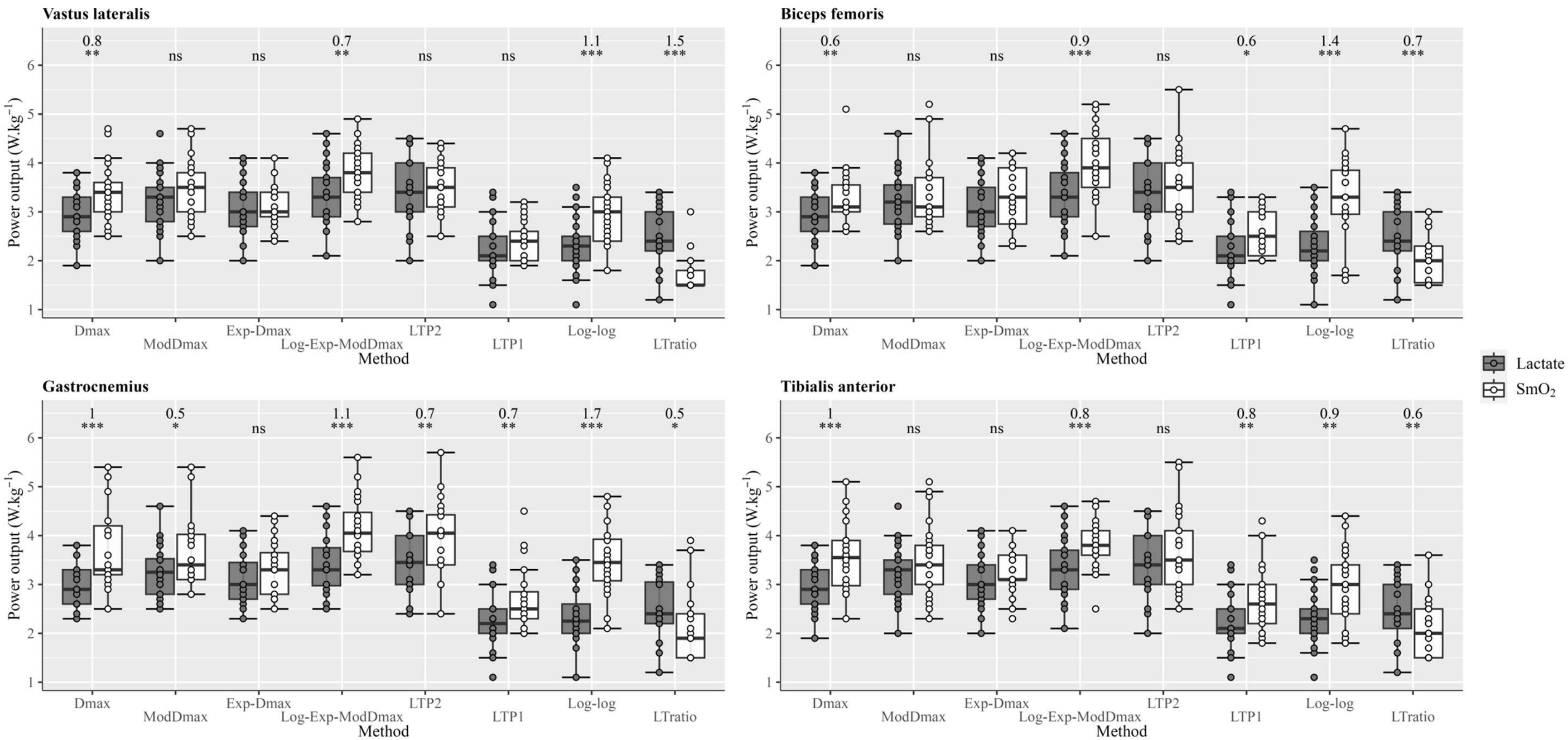

*Fig. 2. Lactate and muscle oxygenation thresholds were obtained using different methods in all participants. Significant differences between durations are identified by symbols (ns non-significant, *p<0.05, **p<0.01, ***p<.001), the value of the effect size being presented above the symbols.*

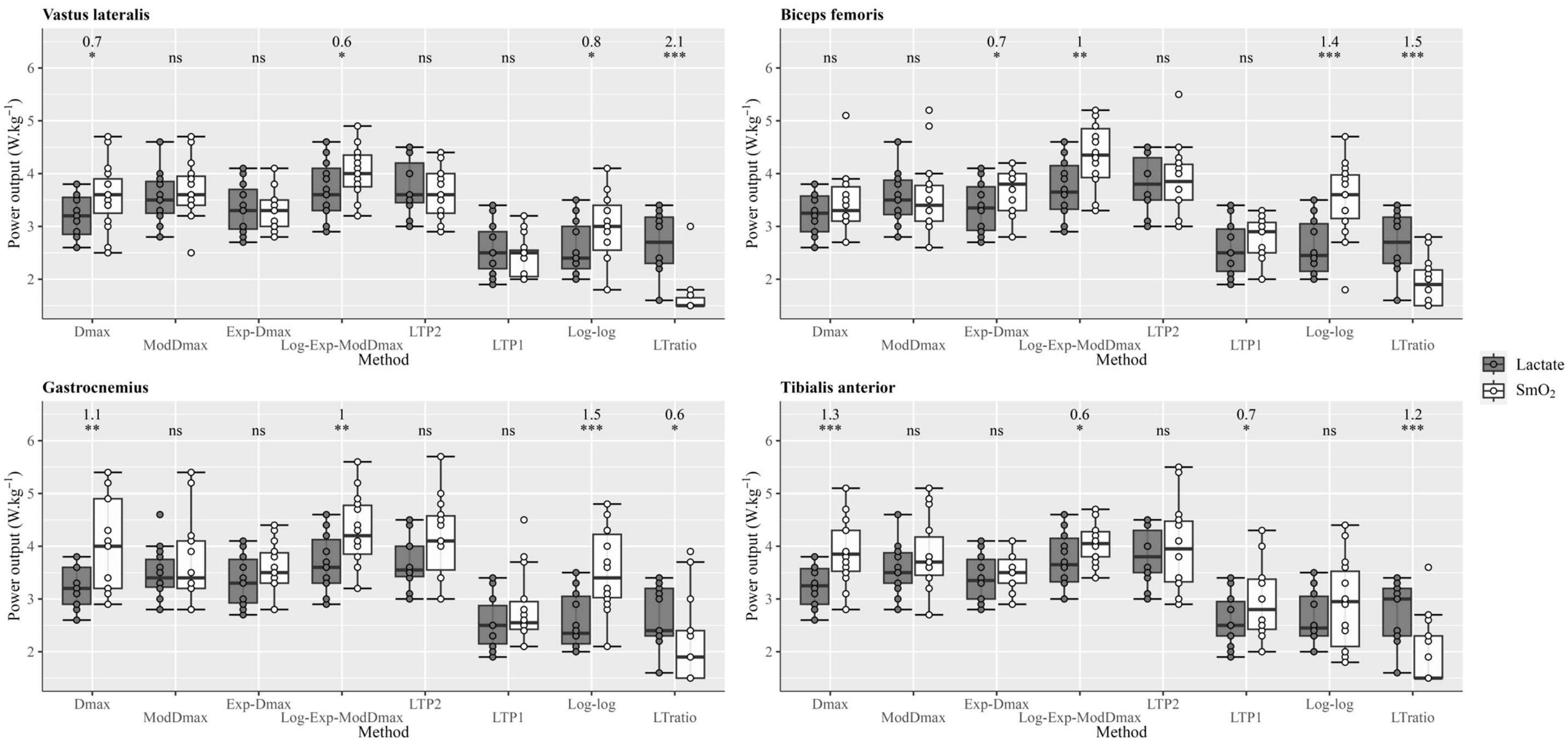

*Fig. 3. Lactate and muscle oxygenation thresholds were obtained using different methods in males. Significant differences between durations are identified by symbols (ns non-significant, *p<0.05, **p<0.01, ***p<.001), the value of the effect size being presented above the symbols.*

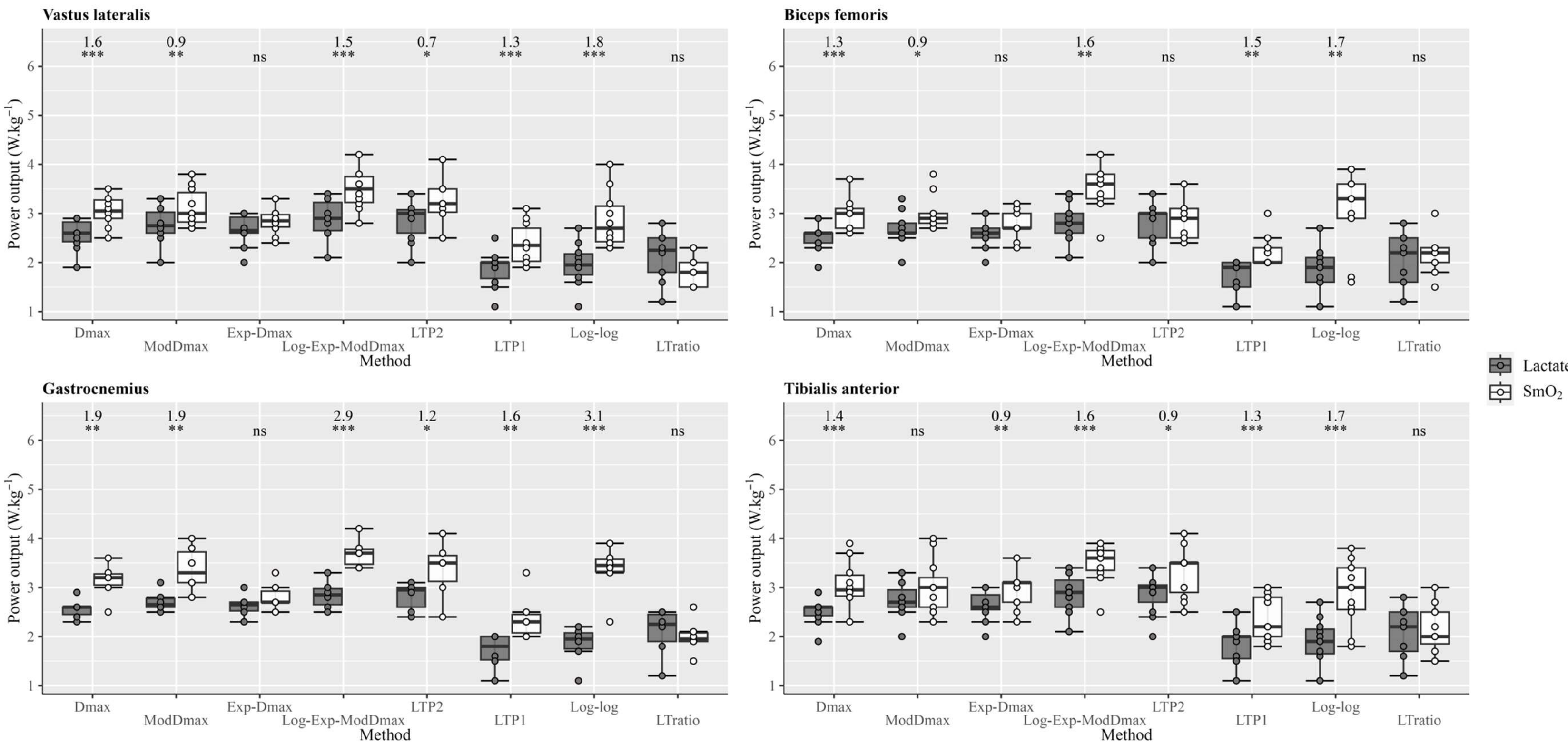

*Fig. 4. Lactate and muscle oxygenation thresholds were obtained using different methods in females. Significant differences between durations are identified by symbols (ns non-significant, *p<0.05, **p<0.01, ***p<.001), the value of the effect size being presented above the symbols.*

Intraclass correlation coefficients

Exp-Dmax was the method with highest ICC in all muscles for all participants (Table 4), being excellent for vastus lateralis (ICC=0.91, CI95%[0.81, 0.96]) and biceps femoris (ICC=0.81, CI95%[0.35, 0.93]), and very good for tibialis anterior (ICC=0.79, CI95%[0.48, 0.93]), and gastrocnemius (ICC=0.80, CI95%[0.45, 0.92]). When the results were divided per sex, Exp-Dmax remained the method with the highest ICC values in males in all muscles (ICC=0.72-0.90). However, for females, the ICC of different methods presented wider CI95%, the differences being in most cases non-significant. Furthermore, the methods used for determining the first threshold (LTP1, Log-log and LTrattio) showed non-significant ICC values in vastus lateralis and tibialis anterior. However, biceps femoris (ICC=0.53, CI95%[0.08, 0.79]) and gastrocnemius (ICC=0.49, CI95%[0.04, 0.77]) presented significant and reasonable results in all participants.

Table 4. Reliability was calculated through the Intraclass Correlation Coefficient (ICC) of the power output threshold in all methods for lactate and muscle oxygenation determination. Confidence Interval at 95% (CI95%). Significant ICC values (p<0.05) are shown in bold letters.

Muscle	Method	All participants			Male			Female		
		p	ICC	CI95%	p	ICC	CI95%	p	ICC	CI95%
Vastus lateralis	LTP1	0,12	0.23	-0.16, 0.56	0.43	0.05	-0.48, 0.54	0.12	0.35	-0.12, 0.77
	LTP2	**<0.01**	**0.57**	0.23, 0.78	**0.03**	**0.46**	-0.03, 0.78	0.09	0.37	-0.15, 0.78
	Dmax	**0.04**	**0.58**	-0.05, 0.84	**0.03**	**0.56**	-0.02, 0.84	0.15	0.26	-0.11, 0.7
	ModDmax	**<0.01**	**0.75**	0.43, 0.89	**<0.01**	**0.80**	0.51, 0.93	0.08	0.40	-0.13, 0.79
	Exp-Dmax	**<0.01**	**0.91**	0.81, 0.96	**<0.01**	**0.90**	0.74, 0.97	**0.03**	**0.76**	-0.05, 0.95
	Log-Exp	**0.03**	**0.63**	-0.03, 0.87	**0.02**	**0.58**	0.05, 0.84	0.13	0.39	-0.07, 0.81
	Log-log	0.33	0.06	-0.17, 0.35	0.60	-0.05	-0.41, 0.4	0.27	0.09	-0.13, 0.48
	LTratio	0.29	0.06	-0.12, 0.31	0.25	0.08	-0.08, 0.37	0.17	0.26	-0.25, 0.72
Tibialis anterior	LTP1	0.20	0.19	-0.25, 0.62	0.20	0.19	-0.25, 0.62	0.27	0.11	-0.18, 0.53
	LTP2	**0.02**	**0.53**	0.02, 0.82	**0.02**	**0.53**	0.02, 0.82	0.06	0.60	-0.1, 0.89
	Dmax	0.12	0.28	-0.12, 0.67	0.12	0.28	-0.12, 0.67	0.13	0.39	-0.09, 0.81
	ModDmax	0.07	0.36	-0.12, 0.72	0.07	0.36	-0.12, 0.72	**0.01**	**0.68**	0.11, 0.91
	Exp-Dmax	**<0.01**	**0.79**	0.48, 0.93	**<0.01**	**0.79**	0.48, 0.93	0.07	0.62	-0.1, 0.91
	Log-Exp	0.14	0.26	-0.2, 0.66	0.14	0.26	-0.2, 0.66	0.16	0.22	-0.11, 0.64
	Log-log	0.97	-0.55	-0.88, 0	0.97	-0.55	-0.88, 0	0.86	-0.19	-0.34, 0.31
	LTratio	0.17	0.18	-0.15, 0.58	0.17	0.18	-0.15, 0,58	0.48	0.02	-0.63 – 0.61
Gastrocnemius	LTP1	**0.02**	**0.49**	0.04, 0.77	**0.03**	**0.45**	-0.02, 0.78	0.17	0.27	-0.1, 0.79
	LTP2	**0.02**	**0.67**	0.03, 0.88	**0.02**	**0.60**	0.03, 0.86	0.11	0.46	-0.12, 0.89
	Dmax	0.07	0.42	-0.1, 0.76	0.10	0.35	-0.12, 0.73	0.51	0.00	-0.2, 0.54
	ModDmax	**0.01**	**0.46**	0.06, 0.74	**0.02**	**0.53**	0.04, 0.82	0.59	-0.04	-0.24, 0.52
	Exp-Dmax	**<0.01**	**0.80**	0.45, 0.92	**<0.01**	**0.72**	0.29, 0.9	0.07	0.54	-0.15, 0.91
	Log-Exp	0.09	0.54	-0.09, 0.85	0.08	0.56	-0.1, 0.87	0.21	0.11	-0.04, 0.58
	Log-log	0.20	0.11	-0.1, 0.4	0.18	0.15	-0.12, 0.52	0.75	-0.05	-0.12, 0.33
	LTratio	0.43	0.04	-0.37, 0.46	0.54	-0.03	-0.48, 0.49	0.53	-0.04	-1.04, 0.77

Biceps femoris	LTP1	**0.01**	**0.53**	0.08, 0.79	0.07	0.35	-0.12, 0.72	0.18	0.20	-0.14, 0.65
	LTP2	**<0.01**	**0.78**	0.55, 0.9	**0.01**	**0.58**	0.07, 0.84	**0.02**	**0.66**	0.03, 0.91
	Dmax	**0.01**	**0.63**	0.1, 0.85	**0.01**	**0.63**	0.14, 0.87	0.16	0.24	-0.15, 0.69
	ModDmax	**<0.01**	**0.61**	0.28, 0.81	**0.03**	**0.53**	0, 0.82	0.16	0.26	-0.22, 0.73
	Exp-Dmax	**<0.01**	**0.81**	0.35, 0.93	**0.04**	**0.74**	-0.05, 0.93	0.19	0.29	-0.34, 0.77
	Log-Exp	0.08	0.62	-0.08, 0.89	0.09	0.60	-0.07, 0.9	0.16	0.25	-0.11, 0.71
	Log-log	0.15	0.16	-0.1, 0.47	0.15	0.21	-0.12, 0.59	0.72	-0.09	-0.29, 0.37
	LTratio	0.79	-0.15	-0.45, 0.23	0.87	-0.22	-0.41, 0.26	**0.03**	**0.59**	-0.02, 0.89

Bland-Altman plots.

Bland-Altman plots were obtained for Exp-Dmax as this was the method with the highest ICC values (Fig. 5). For all participants, the bias of biceps femoris was -0.24 ±0.28 $W.Kg^{-1}$, for gastrocnemius was -0.2 ±0.3 $W.Kg^{-1}$, for tibialis anterior was -0.19 ±0.23 $W.Kg^{-1}$, and for vastus lateralis was -0.06 ±0.19 $W.Kg^{-1}$. Similar biases were observed for males and females.

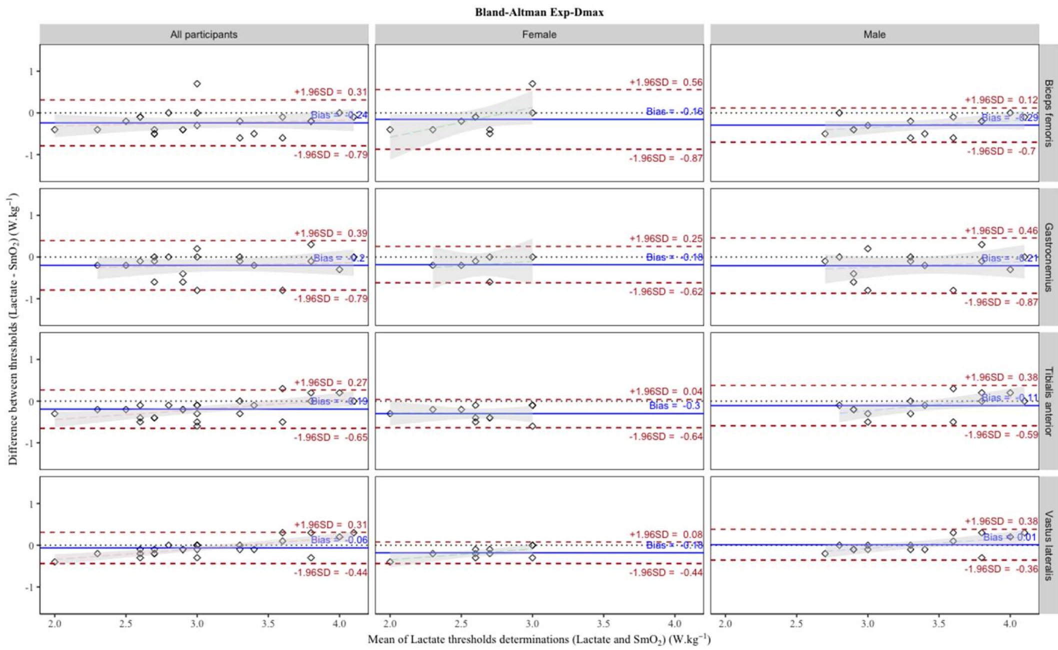

Fig. 5. Bland-Altman plots for the second muscle oxygenation threshold and second lactate threshold expressed in relative power output ($W.Kg^{-1}$). The central continuous blue line represents the absolute average difference between instruments (bias), and the upper and lower red lines represent ± 1.96 standard deviations.

4. Discussion

The purpose of the present study was to compare the identification of the intensity at the first and second thresholds using lactate and SmO_2 data by different mathematical methods in different muscles (vastus lateralis, gastrocnemius, biceps femoris, and tibialis anterior) during GXT. Our main result is that Exp-Dmax presented the highest ICC values in the vastus lateralis, with no differences between lactate and SmO_2 data in determining the threshold value. For the first threshold determination, the ICC values were non-significant in most cases.

The findings of the current study indicate that relying on SmO_2 data to determine the first threshold is not reliable if determined with a commonly used mathematical method

(Jamnick et al., 2020). However, previous studies showed good correlations (r = 0.63) between gas exchange and SmO_2 data at the first threshold in cycling during step tests (van der Zwaard et al., 2016). However, the results of the first threshold were not clearly defined in the study by Feldmann et al. (2022), since both first thresholds are not correlated. The oxygen consumption increase during the first lactate threshold remains linear (Binder et al., 2008) which may explain the difficulty for SmO_2 data to determine this point. Thus, the first ventilatory threshold could be determined by measuring the workload at which blood lactate concentration begin to increase above resting values (Pallarés et al., 2016).

Different mathematical methods to calculate MOT1 and MOT2 (e.g., Dmax, regression double linear and segmented linear regression model) were found in the literature (Cayot et al., 2021; Iannetta et al., 2017; van der Zwaard et al., 2016). Moreover, a previous study suggested that the method used is an important factor in influencing the results, as different correlations were obtained between lactate and SmO_2 data depending on the method (Cayot et al., 2021). For example, Dmax showed a better MOT2 determination than ModDmax as it takes into account the first values which are not considered by ModDmax (Cayot et al., 2021). Our results suggest that the best method to determine the MOT2 is Exp-Dmax, which has previously been suggested for providing a better determination than polynomial methods (Machado et al., 2012). Furthermore, the SmO_2 has an exponential increase above the second threshold similar to blood lactate (Binder et al., 2008). For this reason, it is possible that the ICC was excellent or very good in all muscles. On the other hand, the logarithmic curve method was also used for this analysis, but it requires a greater number of data points. It is worth mentioning that our results are associated with the type of test used, and, for example, in a ramp test, other methods such as LTP2 or regression double linear could be better (Hofmann & Tschakert, 2017; Spencer et al., 2012).

Most of the previous studies assessed one or two muscles using the portable muscle oxygen monitor (Feldmann et al., 2022; Rodrigo-Carranza et al., 2021; Salas-Montoro et al., 2022; van der Zwaard et al., 2016; Yogev et al., 2022) mainly focusing on the vastus lateralis (Feldmann et al., 2022; Rodrigo-Carranza et al., 2021; van der Zwaard et al., 2016) and gastrocnemius (Borges & Driller, 2016; Driller et al., 2016; McMorries et al., 2019). The determination of the MOT2 using mathematical methods was excellent in vastus lateralis in line with the results of Rodrigo-Carranza et al. (2021) who also performed the intervention in cycling. This muscle presented higher ICC values compared to other muscles assessed, which may be explained by different reasons. Although vastus lateralis and biceps femoralis are the muscles most involved during pedaling in cycling presenting a greater neuromuscular activity (Quesada et al., 2015), it is possible that the values of ICC in the biceps femoralis were lower due to their higher proportion of adipose tissue (Niemeijer et al., 2017). Moreover, the ICC was also very good in gastrocnemius, a muscle analyzed in others studies with similar results (Borges & Driller, 2016; Driller et al., 2016; McMorries et al., 2019). Though the biceps femoris and tibialis anterior were not analyzed previously with a portable muscle oxygen monitor, the ICC was very good when including all participants or males. This could mean that, although the vastus lateralis was the muscle most related to the second lactate threshold and, therefore, to systemic changes, the second threshold can also be obtained from other muscles. It is important to take into account that determining thresholds using NIRS seeks to be more localized, and focused on the muscle measured (Perrey & Ferrari, 2018), so the threshold of these muscles may not be so related to the systemic lactate threshold but be more specific. Systemic lactate is highly dependent of the metabolism of the muscles involved in the exercise (Brooks et al., 2022), and for this reason, it is logical to found good agreement between the second systemic lactate threshold and the MOT2 of theses muscles. In this sense, Iannetta et al. (2017) observed how the specific muscle proportion of the quadriceps (vastus lateral, medial and rectus femoris) presented a different hemoglobin signal. Future studies should explore in more detail how the timing of the second threshold varies according to the muscle analyzed.

Different authors have suggested that it is important to measure the adipose tissue thickness (ATT) when NIRS is used during exercise (Niemeijer et al., 2017; Perrey & Ferrari, 2018). Moreover, other authors consider skinfolds greater than 25 mm as an exclusion criterion (van der Zwaard et al., 2016), in our study, any participant had fold values above these skinfold levels. Furthermore, we reduced the ATT risk affecting the measurements by using the SmO_2 variation to calculate MOT with different methods. A recent study observed that measurements using a Humon NIRS device obtained outliers or connectivity problems in participants with high skinfold values (Gandia-Soriano et al., 2022). In our study, females presented, in general, lower ICC values, which could be due to their higher skinfolds (Table 1). Further investigation should explore in more detail how to deal with skinfolds during exercise testing using NIRS.

The main limitation of the present study was that the lactate meter measured the blood lactate concentration, and not the muscle lactate concentration (Green et al., 1983), although some studies have correlated these variables (Cayot et al., 2021; Raleigh et al., 2018; Salas-Montoro et al., 2022). Moreover, the results obtained are specific to the instruments used (Lactate Pro and Moxy) and their uncertainty. Another limitation was not measuring the skinfold in all muscles when the portable muscle oxygen monitors were located, which may well improve the interpretation of the differences between males and females. Aditionally, the blood lactate concentration each 3-min and the SmO_2 was continuosly measured. Thus, future research could explore how the protocol type of GXT (ramp vs. step) could alter reliability in determining the second threshold using SmO_2 data. Finally, although Moxy appeared to have similar reliability to other NIRS devices (Crum et al., 2017), caution should be taken when extrapolating the results to other NIRS devices, as each device has a different inter-optode spacing, sampling rate, signal noise or algorithms to do the calculations (McManus et al., 2018; Perrey & Ferrari, 2018).

The results of the present study suggests that NIRS portable technology presents a practical application in sports training, as it enables the detection of the second threshold during a GXT automatically using the Exp-Dmax method. In comparison to lactate measurements, which require test strips and may result in higher costs, NIRS technology offers a more affordable and convenient solution. Furthermore, NIRS device can be placed on the athlete during the test without causing any discomfort, making it suitable for use in field tests. Notably, when SmO_2 data are focus to detect the second threshold more related with lactate and systemic responses in cycling, the equipment must be positioned in the vastus lateralis muscle. While NIRS technology has shown promise in detecting the second threshold, its use for detecting the first threshold is currently not recommended. Moreover, these results are also interesting for training platforms or apps that detect the MOT2, to implement the Exp-Dmax in their software for this detection. Overall, the practical applications of NIRS technology in sports training are promising, and further research is needed to explore its full potential.

In conclusion, the MOT1 could not be determined using these mathematical methods in the muscles analyzed. However, the MOT2 showed good values in many methods, but the best method in all muscles was Exp-Dmax, and the vastus lateralis was the muscle that presented the best ICC values. Nevertheless, sex should be taken into account when measuring local SmO_2, since skinfolds may affect the determination of thresholds.

Ethical Statement

The study protocol was reviewed and approved by the Ethics Committee of the University of Valencia (No. 2630853), and follows the ethical principles for medical research in humans of the Declaration of Helsinki. Participants were informed of the risks and benefits of participating in the study and signed the informed consent before starting the protocol.

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