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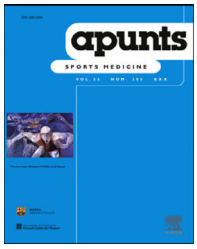

ORIGINAL ARTICLE

Influence of fat percentage on muscle oxygen uptake and metabolic power during repeated-sprint ability of footballers

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Abstract The aim of this study was to analyse the muscle oxygen saturation (SmO_2) dynamics during a repeated-sprint ability (RSA) protocol (8 sprints x 20 meters, 20 s recovery) using near-infrared spectroscopy. Twenty-five footballers were grouped according to the levels of body-fat percentage (level 1: <9%; level 2: 9.1−11.5%; and level 3: >11.6%) from the Spanish third division participated. During RSA, energy cost (EC), metabolic power (MP), speed and total time as external load were measured. Desaturation and resaturation rates and muscular oxygen extraction ($\triangledown$% SmO_2) of the gastrocnemius muscle, along with heart rate (HR) were used as indicators of internal load. $\triangledown$% SmO_2 was identified as the most sensitive variable to detect the minimal change during RSA. Footballers with a lower fat percentage (level 1) achieved a higher $\triangledown$% SmO_2 after the 4th sprint (Δ= −13; p= 0.001) and (Δ= 9.6; p= 0.017) vs level 2 and level 3, respectively. SmO_2 was related to EC (r2= 0.57 p= 0.005), MP (r2= 0.61 p= 0.003), speed (r2= 0.59 p= 0.004) and total time (r2= 0.59 p= 0.004). Therefore, SmO_2 was a better indicator of internal load than HR during RSA. The $\triangledown$% SmO_2 can be used as a parameter to explore potential differences in footballers' RSA performance. Besides, we highlighted the relevance of measuring the body-fat percentage, since it is a variable that affects performance by disturbing $\triangledown$% SmO_2, altering the ability to resist repeated high-speed bouts (sprints), a critical variable in football.

Introduction

In recent years, interest has increased in studying the repeated sprint ability (RSA) of footballers as a fundamental variable in team sports. RSA are characterized by high intensity actions interspersed with short recovery times.[1]

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Considering the actual football competitive demands, players cover more distance at high speed, therefore need better performance to withstand high intensity.[2] Although, RSAs don't usually appear frequently during full-time play due to the high energy demand this implies. RSA can be considered as an independent variable in the soccer training process, as it works as a means to develop acceleration, speed, explosive power of the legs, aerobic power and high running performance.[3] In addition, it directly intervenes in muscle metabolism by improving maximum oxygen uptake, phosphocreatine (PCr) restoration and the glycolytic pathway. This is decisive for the physical performance as it improvement the recovery during sprints.[3] In football, RSA testing is typically characterized by short-distance not exceeding 20 meters, with short recovery periods (<30 seconds). This is important since the combination of very high intensity actions together with insufficient recovery periods generates stresses on muscle metabolic processes[1] and results in the development of neuromuscular fatigue.

When evaluating performance using an RSA, external load indicators such as best time, total time, and fatigue index are often measured. However, to understand the response to muscle energy demands, some internal load parameters such as heart rate (HR) and maximum oxygen consumption (VO_{2max}) should be also monitored. These internal load variables together with the external load variables would give us a better overview of the decrease in RSA due to fatigue accumulation.[4] HR is a physiological variable widely used in football, but HR cannot determine muscle energy capacity during intermittent high-intensity activities such as sprinting and passive recovery.[5] For this reason, the development of theoretical models and software allow knowing other variables as energy cost (EC) and metabolic power (MP), which indirectly assess energy metabolism through the distance covered, speed and accelerometry during training and matches.[6] It has been shown that MP analysis can be useful to adequately assess professional player's performance limitations, especially during high-intensity actions.[6,7] Furthermore, MP represents the required ATP hydrolysis rate to develop the muscular work needed during running.[8]

Non-invasive technology such as near infrared spectroscopy (NIRS) is used to evaluate muscle metabolism, which allows the measurement of muscle oxygen saturation (SmO_2).[9] The interest in evaluating SmO_2 is due to its ability to discriminate metabolic changes within the muscle related to oxygen transport during high-intensity actions. In repeated-sprint activities, it has been proposed to measure the mean rates of desaturation (during the sprint) and resaturation (during recovery between sprints) as indicators of metabolic performance.[10] Furthermore, slower reoxygenation indicates that there is a slower recovery of intramuscular phosphates that are required for high-intensity exercise at levels prior to sprinting exercise lower performance.[10] Besides, some factors such as high values of body fat % and less muscle mass (body composition) affect performance of aerobic and anaerobic capacity, intermittent endurance capacity, RSA, small-side games and football specific activities.[11,12] Likewise, the muscle oxygenation capacity is influenced by adipose tissue,[13] because greater adipose tissue affects muscle oxygen trapping during exercise.

Therefore, we hypothesize that SmO_2 is related to energy cost, metabolic power, heart rate, and performance during RSA. We also consider the body fat % as a variable that intervenes in performance due to its relationship with a better muscle oxygenation capacity and force production. This study aimed to 1) explore the potential relationship between energy cost and metabolic power with SmO_2 dynamics during a RSA; 2) determine the degree of influence of fat percentage on muscle oxygen transport during RSA in footballers.

Methods

Participants

Twenty-five footballers (age 22.4 ± 2.6 years, body weight 74.8 ± 9.9 kg, height 1.81 ± 0.07 m, medial calf skinfold 9.3 ± 2.3 mm, experience 8.5 ± 1.8 years) were assessed. Participants competed in the Spanish third division. The exclusion criterion was that the players did not have at least three months before the measurement a serious illness or disease and a recent musculoskeletal injury that could affect the SmO_2 evaluation. Football players signed the informed consent form and agreed to all protocol risks and benefits of this study. The Institutional Review Board of the University of Extremadura approved the protocol (Registration No.: 131/2018) following the Declaration of Helsinki's principles.

Measures

Repeated-Sprint Ability Test: The RSA test followed guidelines according to the University of Wolverhampton (United Kingdom) and as previously reported.[14] First, the players performed a standardized warm-up as advised by the fitness coach. Players were instructed to run at maximum for each sprint. RSA involved eight × 20 m maximum straight-line sprints followed by a 20 s semi-active recovery period. This protocol was validated and proposed by Aziz et al.[15] Players were instructed to run through the time gates and slow down only after being well away from the photocell instead (Witty, Microgate, Italy). The players then recovered within 10 meters and returned to the nearest line to begin the next sprint.

The electronic photocell gates were adjusted according to the participant's hip height and placed 1.2 m apart between each cell. The tripods instead placed at the zero and 20 m marks and connected to an electronic timer with the sampling rate of 0.01 s. The time for each sprint and total time were evaluated as a performance factor. This protocol demonstrated that the time of total sprint was highly reproducible (Intraclass coefficient, r: 0.98 (95%: 0.96-0.99) and typical error 0.42 sec (95%: 0.32-0.62 s).[15]

Energy Cost and Metabolic Power: EC and MP were evaluated based on speed, as referenced in other studies that use GPS,[6,16] but it can be exported when using photocell gates that are direct measurements. The energy cost (EC in J/ kg/ m) and MP (in W/kg) were evaluated during both situations using the first algorithm of Osgnach et al., (2010) According to this approach, the energy cost can be defined as:

$$\mathbf{EC} = (\mathbf{155.4} \cdot \mathbf{ES5} - \mathbf{30.4} \cdot \mathbf{ES4} - \mathbf{43.3} \cdot \mathbf{ES3} + \mathbf{46.3} \cdot \mathbf{ES2} + \mathbf{19.5} \cdot \mathbf{ES} + \mathbf{3.6})\mathbf{EM} \cdot \mathbf{KT} \quad (1)$$

where EC is the energy cost of accelerated running on flat terrain (J/kg/m), ES is the equivalent slope equal to ES=tan

[90° − arctan(g/af)] with g is the gravity acceleration (9.81 m/s2). af is the forward acceleration. EM is the equivalent body mass equal to [(af ^{2}/g^2)+1]0.5, and KT is the constant considering the characteristics of a grass field (1.29).

Once the EC is known, the MP at any given moment can be easily obtained as follows:

$$\mathbf{MP} = \mathbf{EC} \cdot \mathbf{v} \quad (2)$$

Heart Rate zones: First, the resting heart rate was obtained after 10 minutes of resting in the supine position (Polar T31, Kempele, Finland). Then the mean heart rate value in bpm was obtained during each sprint. Finally, the HRmax was calculated using the 208-0.7*age formula.[17]

The calculation of the training zones was based on the HR reserve percentage (% HRres) using the following formula: HRres = (the coincidence of the mean HR - HR at rest) / (HRmax - HR at rest) x 100.[18]

Muscle Oxygenation Dynamics: Measurements were carried out with the portable NIRS sensor device with a sampling rate of 1 Hz (MOXY, Fortiori Design LLC, Minnesota, USA). NIRS uses the modified Beer-Lambert law to determine micromolar changes in tissue oxyhemoglobin, deoxyhemoglobin, and total hemoglobin using differences in light absorption characteristics at 750 and 850 nm, calculated in terms of the SmO_2 [expressed in % and calculated as oxyhemoglobin / (oxyhemoglobin + deoxyhemoglobin) × 100)]. The data were averaged based on 1 s, and a moving average (3 s) was applied to smooth the signal with the Golden Cheetah (GoldenCheetah version 3.4, USA). The raw SmO_2 signal was treated with a soft spline filter to reduce the noise created by movement.[19] Using a Minitab 16 Statistical Software (2010). Additionally, real-time data monitoring (visible only to researchers) was used using the software via ANT+ technology.

Muscle oxygenation dynamics analysis was performed in the gastrocnemius medialis (GM). GM represents a good aerobic capacity measured with NIRS [20] The device was connected with adhesive tape and wholly covered with a neoprene sleeve. Skinfold thickness was measured between the emitter and the detector using a skinfold caliper (Harpenden calipers, British Indicators, Hertfordshire, UK) to assess skin thickness and adipose tissue of GM. The GM skinfold thickness (9.3 ± 2.3 cm) was less than half the distance between the emitter and the detector in all cases. To calibrate and normalize values, the SmO_2 on a functional scale of 0-100%, arterial occlusion method (AOM). The arterial occlusion tourniquet was placed on all participants' dominant leg at the thigh level following the previous guidelines.[21] The tourniquet was performed with a pneumatic tourniquet (Rudolf Riester GmbH, DE) and remained inflated at >300 mmHg for 6 min to find the minimum SmO_2 (SmO_2min) and was determined through the average of 20 to 10 visible points in the plateau, after 6 min. The tourniquet was released, and an additional 3 min of measurement was performed to evaluate the hyperaemia response and find the maximum oxygen value (SmO_2max) in 10 or 5 data points at the end after AOM.

Before the start of RSA, the subjects stopped for 30 s, during which the SmO_2 reference was established. For the RSA analysis, well-differentiated phases were identified: (a) the execution phase (phase 1), where a desaturation process was observed, represented by a downward slope; (b) a recovery phase (phase 2), where a re-saturation process was observed, characterized by an upward slope.[10]

For the analysis of muscle oxygen saturation dynamics, the following variables were calculated:

1. The desaturation rate, understood as the difference between the maximum (work interval) and minimum SmO_2 values (rest interval) and divided by the duration of the work interval. Similarly, the re-saturation rate was defined as the difference between the minimum (rest interval) and maximum values of SmO_2 (working interval), divided by the rest duration of the interval (20 s).
2. The percentage of SmO_2 decrement from the desaturation and re-saturation values were obtained during each sprint using the difference between SmO_2 at the start and end of the sprint. The SmO_2 start value was considered 1 s before starting each series, while the SmO2 stop value was determined in the last second of the work interval of each sprint with the following formula:

$$\nabla\%\mathbf{SmO_2} = [(\mathbf{SmO_2Stop} * \mathbf{100}/\mathbf{SmO_2start}) - \mathbf{100}] * -\mathbf{1}$$

▽% SmO_2 should be interpreted as the % muscle oxygen extraction capacity from the anaerobic level.[10]

Body composition: A digital scale (Seca 225kg, Germany) and a portable stadiometer (Seca 220, Germany) were used to calculate body mass and height, respectively. The BMI was determined through height and weight according to the standard procedure (BMI= weight/height2, kg · m- 2). The same researcher measured subcutaneous fat in skinfolds and weight and height in the morning. Six subcutaneous fatty skinfold sites (abdominal, subscapular, iliac crest, calf, and thigh) were measured with a skinfold caliper (Harpenden, Mediflex, Iceland, NY, USA) on the left side of the body, following the recommendations of the International Biological. The value obtained at each site was the mean of three valid measurements.[22] The sum of the six skinfolds and the percentage of the fat mass was calculated/assessed using the six subcutaneous fatty skinfolds Yuhasz, 1974:

$$\%\mathbf{Fat\ mass} : (\Sigma\ \textit{skinfolds} * \mathbf{0.097}) + \mathbf{3.64}.$$

Later, different fat percentage groups were established (level 1= <9 %; level 2= 9.1 to 11.5%; and level 3= >11.6%) (see Fig. 1), which can be approximated to level 1= highly trained soccer players, level 2= competitive level footballers and level 3= amateur footballers.[23]

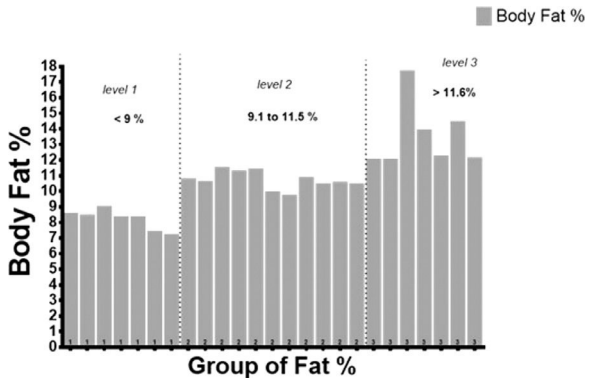

Fig. 1 Group differences based on body fat % level. Groups sample (level 1: n= 7; level 2: n=11 and level 3: n=7).

Table 1 Descriptive variables of body composition and SmO_2 in footballers

Variables	Media DS
Weight (kg)	74.8 ± 9.9
Body Mass Index	22.5 ± 2.0
Fat %	9.2 ± 2.0
Fat Mass (kg)	8.1 ± 2.6
Muscle mass (kg)	33.2 ± 3.6
Skinfold gastrucnemius (mm)	9.3 ± 2.3
SmO_2 (%) Desaturation	29.2 ± 14.6
SmO_2 (%) Reoxygenation	34.5 ± 15.9

Design and procedures

This study followed a cross-sectional observational design aiming to relationships between RSA and SmO_2 studied, using physiological response of HR, %HR and mechanical responses based on time and individual speed during RSA. All tests were carried out on a heated sports court with an ambient temperature of 16−19 °C and relative humidity of 40%−50%, respectively. Data were obtained during the preseason. RSA stimuli were performed with the following criteria to avoid possible biases: a) a minimum of 48 hours of rest, after the last training was a recovery load [i.e., evaluations were carried out on Tuesday or Wednesday, depending on the day a game was played (Saturday or Sunday)], and the test was carried out before training to try to guarantee maximum recovery and (b) participants were instructed not to consume alcohol or caffeine 24 h before each test and maintain habitual sleep habits to avoid a decrease in performance. The participants were divided into six workgroups to guarantee considerable measurement time for each player.

Statistical

A descriptive analysis of the variables was performed during the test expressed as mean ± SD. Analysis of variance, followed by Tukey post hoc tests, was used to compare the eight sprints of the RSA test. This approach allowed us to make comparisons between the baseline (Second Sprint) and the other sprints. This is due to the fact that the first sprint is always different from the other sprints, because an intramuscular pressure occurs that abruptly decreases the SmO_2. Measurement error was expressed in "typical percentage error" (TE) and "minimum detectable change" (MDC). The typical error was calculated by dividing the SD of the difference score by $\sqrt{2}$. This specific percentage error is a coefficient of variation and is considered highly reliable if less than 5%. MDC values [also referred to as the "smallest detectable difference (SMD)], which reflects the magnitude of change necessary to provide confidence that the change was not resultant of random variation or measurement error, were calculated as $1.96*\sqrt{2}*TE$.[24] Then, a comparative analysis of the mechanical variables, HR% and $\bigtriangledown$% SmO_2 is applied for the three groups of fat percentage (level 1 = <9%; level 2 = 9.1 -11.5, level 3 => 11.6).

Table 2 Workload response during repeated-sprint in footballers

Variables	Time (s)	Speed (m/s)	Energy Cost J.kg.m^{-1}	Metabolic Power W.kg^{-1}	Heart Rate (bmp)	Heart Rate reserve (%)	Desaturation rate	Re-saturation Rate	%V SmO2
Sprint 1	$3,48 \pm 0.23$	5.04 ± 0.38	3.53 ± 0.65	20.6 ± 5.13	134 ± 11	54 ± 7	$-0,25 \pm 3.13$	0.05 ± 0.55	-8.9 ± 39.2
Sprint 2	3.33 ± 0.33 [a]	5.07 ± 0.58 [a]	4.14 ± 1.12 [a]	25.7 ± 9.2 [*a]	145 ± 1 [*a]	63 ± 10 [*a]	1.29 ± 1.33	-0.20 ± 0.26	10.6 ± 14.9 [a]
Sprint 3	3.38 ± 0.23	5.00 ± 0.42	3.84 ± 0.74	23.1 ± 6.0	154 ± 13 [*a,b]	70 ± 9 [*a,b]	1.91 ± 1.30 [a]	-0.31 ± 0.20	20.5 ± 14.4 [*a]
Sprint 4	3.49 ± 0.23 [b]	4.88 ± 0.40 [b]	3.51 ± 0.75	20.5 ± 6.1	158 ± 12 [a,b]	73 ± 9 [a,b]	2.16 ± 1.57	-0.37 ± 0.26 [a]	24.4 ± 15.6 [*a]
Sprint 5	3.58 ± 0.26*	4.92 ± 0.37	3.26 ± 0.61 [*b]	18.4 ± 4.7 [*b]	162 ± 12 [a,b]	76 ± 8 [a,b]	1.63 ± 1.34	-0.29 ± 0.23 [a,b]	18.0 ± 10.7 [a]
Sprint 6	3.46 ± 0.24	4.80 ± 0.40*	3.60 ± 0.70	21.1 ± 5.63	163 ± 11 [a,b]	77 ± 8 [a,b]	1.78 ± 1.48	-0.30 ± 0.24	19.4 ± 14.3 [*a]
Sprint 7	3.37 ± 0.28	4.85 ± 0.49*	3.93 ± 0.90	23.9 ± 7.2	165 ± 11 [a,b]	78 ± 8 [b]	1.65 ± 1.22	-0.27 ± 0.19	18.2 ± 12.7 [a]
Sprint 8	3.62 ± 0.31*	4.81 ± 0.49*	3.21 ± 0.78 *	18.2 ± 6.1*	164 ± 12 [a,b]	78 ± 9 [a,b]	2.10 ± 1.19 [a]	-0.38 ± 0.19 [*a,b]	23.5 ± 12.4 [*a b]
MDC	**0.18 (5%)**	**0.21 (4%)**	**0.53 (15%)**	**5.5 (24%)**	**7 (4%)**	**4 (5%)**	**1.05 (58%)**	**0.18 (-70%)**	**8 (49%)**
SEM	**0.06 (2%)**	**0.07 (2%)**	**0.19 (5%)**	**1.9 (9%)**	**2 (1%)**	**1.5 (2%)**	**0.38 (21%)**	**0.06 (-25%)**	**3 (17%)**

Pos hoc: a= difference with sprint 1; b= difference with sprint 2 and * difference using the MDC between the first three sprints

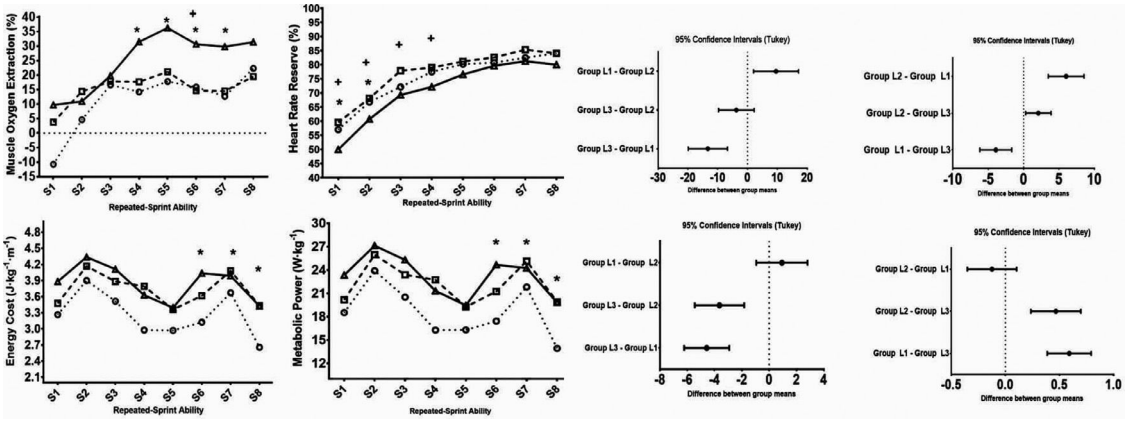

Fig. 2 Influence of body fat % in the response of muscle oxygen extraction, heart rate, energy cost and metabolic power during the sprint-repetitions.
Note: 1) RSA test graphs: a) muscle oxygen extraction, b) heart rate, c) metabolic power and d) energy cost. 2) 95% confidence interval: a) muscle oxygen extraction, b) heart rate, c) metabolic power and d) energy cost.
Difference between low body fat % (level 1) vs higher body fat % (level 2 and level 3), (*) p value <0.05 statistically significant. Difference between the body fat % (level 2) vs the body fat % (level 3), (+) p value <0.05 statistically significant.

Furthermore, to analyze the influence of the Muscle Oxygenation variables with variables of EC, MP, and performance, Multiple regression (Stepwise) analyses were performed. This was determined by the researcher, based on the factor within-subjects (behavior of the variable), Pearson's correlation coefficient >0.50, and a significance p-value of <0.05 (Supplementary Table 1), along with this the percentage of prediction between variables with R^2. Data were analyzed using IBM SPSS Statistics V.22.0.

Results

Table 1 shows the descriptive values of the sample, represented by the mean and standard deviation. Table 2 represents the mean $\pm$ standard deviation for each of the sprints. First, differences are observed between the first and second sprints in all the external load variables (Time, Speed, Energy Cost, and Metabolic power). Likewise, the HR obtained changes in each of the sprints. And the desaturation rate there were only changes between 1st sprint and 3rd sprint (Δ= 2.17 p= 0.035) and 8 (Δ= 2.40 p= 0.010), the re-saturation rate between 1st sprint and 3rd, 4th, and 8th sprints and the $\bigtriangledown$%SmO_2 with between 1st sprint and all other sprints.

Changes from 2ndsprint were also observed to identify the important points, where we found a decrease in performance (time and speed) from sprint 4 (Δ= -2.53 p= 0.018) and (Δ= 0.12 p= 0.021) respectively. Likewise, the decrease in EC (Δ= 0.88 p= 0.025) and MP (Δ= 7.23 p= 0.027) occurs from the 5th sprint. Regarding the SmO_2 variables, they were identified in the re-saturation rate at the 5th sprint (0.166-0.024) and at the 8th sprint (Δ= 0.174 p=0.030) and the $\bigtriangledown$%SmO_2 at the 8th sprint (Δ= -12.92 p= 0.028), all in compared to the second sprint.

Also, considering that all subjects responded to the MDC, we found that performance decreases from the 5th sprint to the last sprint, and its associated speed begins to drop from the 6th sprint. Regarding SmO_2 variables, the MDC of the reoxygenation rate was only at the 8th sprint and the oxygen extraction ($\bigtriangledown$%SmO_2) at the 4th and 8th sprint. The response was highly variable for the SmO_2, and there is no clear answer about the most significant changes. Likewise, in

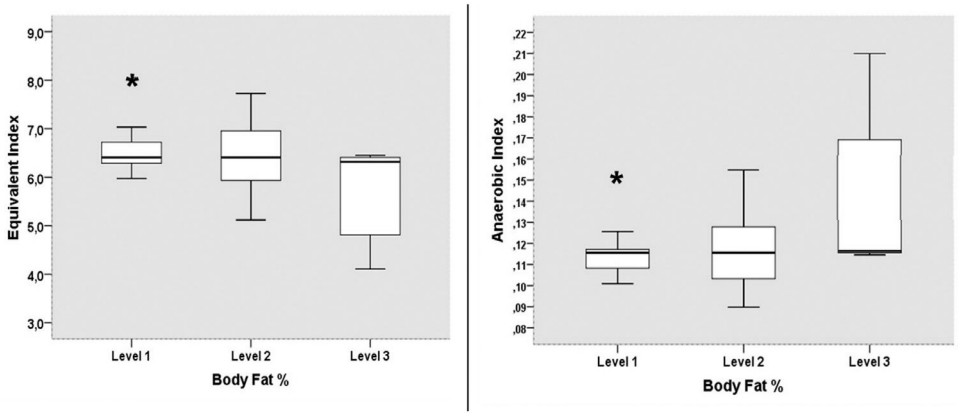

Fig. 3 Differences of equivalent Index and Anaerobic Index by groups of body fat % levels.

Table 3 Performance prediction based on heart rate and muscle oxygen saturation, and using body fat percentage as a relevant variable during RSA.

Variables Body Fat %	External Load		Perfomance	
Internal Load-Physiological	Energy Cost J.kg. m^{-1}	Metabolic Power W.Kg $^{-1}$	Speed (m/s)	Total time (sec)
%V SmO2	r= 0.76 r2=0.57 SE= 0.31	r= 0.78 r2=0.61 SE= 2.56	r= 0.79 r2=0.59 SE= 0.31	r= 0.77 r2=0.59 SE= 0.77
Desaturation rate	F= 7.94 p= 0.005	F= 7.84 p= 0.003	F= 8.18 p= 0.004	F= 6.93 p= 0.004
Re-saturation Rate	(k) 3.610	(k) 21.320	(k) 5.793	(k) 27.794
	0.015	0.117	0.008	-0.040
	3.165	25.558	1.732	-8.013
	19.162	154.788	10.478	-48.442
Heart Rate (ppm)	r= 0.43 r2=0.18 SE= 0.42	r= 0.43 r2=0.19 SE= 3.43	r= 0.40 r2=0.16 SE= 0.23	r= 0.38 r2=0.15 SE= 1.09
Heart Rate Reserve (%)	F= 1.16 p= 0.223	F= 1.72 p= 0.211	F= 1.45 p= 0.264	F= 1.32 p= 0.294
	(k) 0.454	(k) -4,311	(k) 4.095	(k) 35.570
	0.070	0.568	0.037	-0.165
	-0.106	-0.871	-0.055	0.246

terms of heart rate, all the subjects responded in the first three sprints, then there are no significant changes due to entering high-intensity zones.

Fig. 2 shows the evolution of oxygen extraction, heart rate, energy cost, and metabolic power during RSA. The results were divided into a group of body fat %. First, in relation to oxygen extraction ($\bigtriangledown$%SmO_2), from the 4th sprint, greater oxygen extraction is observed with lower levels of body fat: Tukey's multiple comparisons between Group level 1 vs. Group level 3 (Δ= -13; 95% CI= -20 to -6, p= 0.014) and Group level 1 vs. Group level 2 (Δ= 9.6; 95% CI= 2.0 to 17; p= 0.018).

Heart rate shows a difference in the first sprints: Tukey's multiple comparisons between Group level 1 vs. Group level 3 (Δ= -3.9; 95%CI= -6.2 to -1.7; p= 0.003), Group level 2 vs. Group level 3 (Δ = 2.1; 95%CI= 0.29 to 3.9; p= 0.026) and Group level 2 vs. Group level 1 (Δ= 6.0; 95% CI= 3.5 to 8.5; p= 0.000) from the 5th sprint there are no differences between groups where the high intensity zones relative to HR reserve % begins.

Regarding EC and MP, the difference is observed in the 6th, 7 and 8 sprint: Tukey's multiple comparisons between Group level 3 vs. Group level 1 (Δ = -4.6 95% CI = -6.2 to -2.9 p = 0.000) and Group level 3 vs. Group level 2 (Δ = -3.6 95% CI = -5.4 to -1.8 p = 0.001).

Fig. 3 presents the box-and-whisker plot of the equivalent index's mean values and anaerobic index through the body fat % groups. In graph a) a difference of the equivalent index is observed between-group level 1 vs group level 3 (Δ = 1.01 p = 0.034) and in graph b) that represents the anaerobic index differences were found between group level 1 vs group level 3 (Δ = -0.06 p = 0.043).

Table 3 represents the multiple linear regression explanation models for external load and performance based on SmO_2 and heart rate variables. It is shown that SmO_2 better predicted energy cost, metabolic power, speed, and total time than heart rate during the test, with a prediction percentage of 57% (EC), 61% (MP), 59% (Speed) and 59% (TT sec).

Discussion

This is the first study that related SmO_2 dynamics with variables of energy cost and metabolic power associated with speed. %Body fat stands out as a contaminating performance variable that can influence the ability of muscle oxygen extraction to maintain high speed during RSA.

Regarding Table 2, it indicates that the performance decrease in EC, MP, total time, and muscle oxygenation begins from the 4th and 5th sprint, results are similar to previous studies,[10] this is due to the time factor (> 1 m) of the test as a causative agent of fatigue and decrease in PCr and glycolytic pathways[25,26] necessary to satisfy the energy demand of the sprint. Also, during the first sprints there is a decrease of Intramuscular oxygen partial pressure (IPO_2), which causes immediate desaturation. Then the oxidative phosphorylation pathway begins and an increase in blood flow due to the hyperemic response; this phenomenon is associated with the ability to remove and buffer H+ ions and the ability to replace intramuscular PCr.[27] Interestingly, these HR stages no longer detect the MDC and have begun a slow increase in VO_2 and HR kinetics due to the high-intensity. Likewise, in $\bigtriangledown$%SmO_2 a decrease in the values is observed as the test progresses compared to the 1st sprint. Other studies have explored the communication between the decline in SmO_2 and the increase in HR as a function of athletes' speed.[28,29] However, they do not interpret muscle oxygenation with changes in metabolic pathways because HR discriminates the training zones, but SmO_2 decreases and increases with movement, regardless of slow HR kinetics during high-intensity exercise.[10,28] This is one of the problems of interpretation of data variability, and this does not allow an adequate analysis of performance in scientific studies; authors have discarded it as a performance factor.[32] while other authors promote it as an observable variable product of the impact of speed.[30–32] In this regard, our research found that SmO_2 variables better predicted performance: EC, PM, and total time. However, our study used the MDC, with which we can know athletes respond to the true

change (%) in each of the sprints. The $\bigtriangledown$% SmO_2 was observed as the most sensitive parameter, where we found differences in each sprint and not in the desaturation rate and re-saturation rate as previously described.[10]

Consequently, we were able to observe in Fig. 2 a greater $\bigtriangledown$% SmO_2 with a lower body fat, (<9% fat) from the 4th sprint compared to group levels 2 and 3 (>9% fat). This can be explained for two reasons: 1) it has been observed higher SmO_2 values in subjects with greater adipose tissue,[13] however this is not very likely, because the sample obtained a mean calf skinfold 9.3 mm (see Table 1), which is considered low and with good reproducibility and sensitivity for measurements with the MOXY instrument[33] and 2) the activation of type II fibers is better in high trained subjects with less body fat %[34] in addition, have a greater capacity to extract oxygen through the glycolytic pathway, and not the type I fibers that depend more of the HHB or increase SmO_2 that indicates the "oxidative phosporylation",[35–37] this is more likely to have occurred in our study, because similarly, together with the extraction of muscle oxygen, a better performance was obtained in the equivalent index and anaerobic index (See Fig. 3). In general, anaerobic systems can produce high metabolic power but have limited energy capacity. In contrast, the aerobic system has a large capacity, but is characterized by considerably less peak power and inertia.[38]

In this same context, lower HR values were observed in group level 1 (body fat %), this means that a greater $\bigtriangledown$%SmO_2 by glycolytic route compensates the cardiac output,[39] studies such as Casazza et al.,[40] and Vasquez-Bonilla et al.,[41] support this foundation since subjects with a lower body fat can metabolize ATP energy faster through the anaerobic metabolism (glucose) in a short time; this also delays the activation of type I fibers that are dependent on oxygen and increase the blood flow[42] at the end of the RSA tests.[10] However, without measurements of the contributions of the energy pathways,[43] it is difficult to know the performance of one metabolic substrate or another.

Finally, for the performance prediction employing SmO_2, it depends on the body fat % as a variable that contaminates performance; heart rate may not have much relevance in interpreting the decrease in EC, and MP is currently used. Recent studies such as that of Paquette et al., (2018) highlighted the importance of using peripheral adaptations during short and long events since they were able to predict performance through muscle oxygenation by multiple regression analysis. The $\bigtriangledown$% SmO_2 with NIRS is a predictor of metabolic performance that approximates VO_2max and energy expenditure. However, we suggest that with portable NIRS we will obtain better results in the information when interpreting the physiological adaptations of peripheral form and fatigue resistance.[12] However, we need more studies as in real game situations.

Limitations

This study must be seen in the light of some limitations. The main limitation was the lack of information regarding movement tracking monitoring to detect changes in acceleration and deceleration during the tests, especially at 5–10 meters; and the measurement of the resting VO_2 and lactic acid, which are factors that influence capacity of muscle oxygenation. Also, we could explore mechanisms related to fatigue of the central nervous system that may have an important influence with energy systems to determine the interaction of SmO_2 dynamics with EC and MP during the high intensity shown in the RSA.

Conclusion

Considering the results of this study, it's proposed to measure SmO_2 dynamics and $\bigtriangledown$% SmO_2 as a physiological variable for to find potential differences in sport performance, because when the energy cost and metabolic power begins to decrease, so does the increase in muscle oxygen extraction during repeated sprints in footballers.

Therefore, SmO_2 seems more promising than %RH as an indicator of internal load during high-intensity running. In addition, we address the influence of having a low body fat % during the season, since it greatly influences the players speed, together with the ability to oxygenate the muscles.

Ethics approval

The study design was approved by the Bioethical and Biosecurity Commission of the University of Extremadura (Reg. Code 131/2018).

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Data available statement

The data that support the findings of this study are available on request from the corresponding author, [initials]. The data are not publicly available due to [restrictions e.g. their containing information that could compromise the privacy of research participants].

Conflicts 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.

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