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Original

Relationship between anaerobic work capacity and critical oxygenation in athletes

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ABSTRACT

Objective: Anaerobic work capacity (AWC) is understood as the maximum power that the athlete can withstand over time, conditioned by high intensity effort and it is important to interpret it for the performance improvement. In addition, the muscle oxygen saturation (SmO_2) provides information on muscle metabolism and hemodynamics. Likewise, critical oxygenation (CO) is the highest metabolic rate that results in a fully oxidative energy supply that reaches a stable state at the substrate level. The main problem is that SmO_2 generally offers a traditional laboratory interpretation without application in field tests, Therefore, the purpose of this study is to provide the use of CO as an indicator of AWC performance in high intensity exercise.
Methods: Twenty-two male rugby players participated. Peak torques during an isokinetic fatigue test and muscle oxygen consumption (mVO_2) and SmO_2 in the vastus lateralis were measured. A correlation and multiple regression analysis were applied to find an explanatory prediction model of the AWC.
Results: A greater SmO_2 amplitude and CO would mean less anaerobic work (r = -0.58 and r=-0.63) and less force production. In addition, CO along with weight (kg) can explain the AWC by 64% during high intensity exercise.
Conclusion: The measurement of critical oxygenation is associated with the AWC, so should be considered a performance factor. These parameters could be included in NIRS sensors to evaluate muscle metabolism.
Keywords: Athletic performance; Skeletal muscle; Oxygen consumption; Anaerobic threshold; Energy metabolism; Regional blood flow.

Relación entre la capacidad de trabajo anaeróbico y la oxigenación crítica en deportistas

RESUMEN

Objetivo: La capacidad de trabajo anaeróbico (AWC) se entiende como la potencia máxima que el deportista puede soportar a lo largo del tiempo, condicionada por un esfuerzo de alta intensidad y es importante interpretarla para la mejora del rendimiento. Además, la saturación de oxígeno muscular (SmO_2) proporciona información sobre el metabolismo muscular y la hemodinámica. Asimismo, la oxigenación crítica (OC) es la tasa metabólica más alta que da como resultado un suministro de energía completamente oxidativo que alcanza un estado estable a nivel de sustrato. El principal problema es que SmO_2 generalmente ofrece una interpretación de laboratorio tradicional sin aplicación en pruebas de campo, por lo tanto, el propósito de este estudio es proporcionar el uso de OC como indicador del rendimiento de AWC en ejercicio de alta intensidad.
Métodos: Participaron 22 jugadores masculinos de rugby. Se midieron los picos máximos después de una prueba de fatiga isocinética y el consumo de oxígeno muscular (mVO_2) y SmO_2 en el musculo vasto lateral. Se aplicó un análisis de correlación y regresión múltiple para encontrar un modelo de predicción explicativo del AWC.
Resultados: Una mayor amplitud de SmO_2 y OC supondría un menor trabajo anaeróbico (r = -0,58 y r=-0,63) y una menor producción de fuerza. Además, el CO junto con el peso (kg) pueden explicar el AWC en un 64 % durante el ejercicio de alta intensidad.
Conclusión: La medición de la oxigenación crítica está asociada a la AWC, por lo que debe considerarse un factor de rendimiento. Estos parámetros podrían incluirse en sensores NIRS para valorar el metabolismo muscular.
Palabras claves: Rendimiento atlético; Músculo esquelético; Consumo de oxígeno; Umbral anaeróbico; Metabolismo energético; Flujo sanguíneo regional.

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Relação entre capacidade de trabalho anaeróbico e oxigenação crítica em atletas

RESUMO

Objetivo: A capacidade anaeróbica de trabalho (AWC) é entendida como a potência máxima que o atleta pode suportar ao longo do tempo, condicionada por um esforço de alta intensidade, sendo importante interpretá-la para melhorar o desempenho. Além disso, a saturação muscular de oxigênio (SmO_2) fornece informações sobre o metabolismo muscular e a hemodinâmica. Da mesma forma, a oxigenação crítica (OC) é a taxa metabólica mais alta que resulta em um suprimento de energia totalmente oxidativo atingindo um estado estável no nível do substrato. O principal problema é que o SmO_2 geralmente oferece uma interpretação laboratorial tradicional sem aplicação em testes de campo, portanto, o objetivo deste estudo é fornecer o uso do CO como indicador de desempenho de AWC em exercícios de alta intensidade.

Métodos: Participaram 22 jogadores de rugby do sexo masculino. Foram medidos os picos máximos após um teste de fadiga isocinética e o consumo de oxigênio muscular (mVO2) e SmO_2 no músculo vasto lateral. Uma análise de correlação e regressão múltipla foi aplicada para encontrar um modelo explicativo de predição do AWC.

Resultados: Uma maior amplitude de SmO_2 e CO implicaria em menor trabalho anaeróbio (r = -0,58 er = -0,63) e menor produção de força. Além disso, o CO junto com o peso (kg) pode explicar a AWC em 64% durante o exercício de alta intensidade.

Conclusão: A medida oxigenação crítica prevê AWC, portanto, deve ser considerada um fator de desempenho. Esses parâmetros podem ser incluídos em sensores NIRS para a medição do metabolismo muscular.

Palavras-chave: Desempenho atlético; Músculo esquelético; Consumo de oxigênio; Limiar anaeróbio; Metabolismo energético; Fluxo sanguíneo regional.

Introduction

The Anaerobic work capacity (AWC) is understood as the maximum power that the athlete can withstand over time, conditioned by high intensity effort.[1] Based on the maximum effort and duration of the exercise, the AWC is considered as an "oxygen independent" energy system where the phosphate and glycolysis pathway predominate, and to a lesser extent the oxidative phosphorylation.[2,3] The AWC performance is present in exercise modalities such as intermittent sprinting, maximum speed, repeated jumps and maximum strength exercises. In addition, AWC is related to the ability to oxygenate the muscles during high intensity exercise, due to the phosphocreatine (PCr) degradation process that is necessary to produce power.[4] So we have the possibility to predict the AWC over time through the amount of muscle oxygen consumption in athlete.[5]

Since the end of the 1980s to present, near infrared no-invasive (NIRS) technology has been used to assess the oxidative capacity of skeletal muscle through the consumption of muscle oxygen (mVO_2) during exercise and in resting activities. The way it is generally measured is based on the differences in oxygenated hemoglobin ([O_2Hb] the one that is inside the muscle) and deoxygenated hemoglobin (Hbb) the one that the muscle uses).[6] However, the most commonly used NIRS-derived variable in field tests is muscle oxygen saturation (SmO_2).[7] SmO_2 reflects the dynamic balance between O_2 supply and O_2 consumption and is independent of the path of the near infrared photon in muscle tissue; its values range from 1% to 100%. There are several portable NIRS devices that use SmO_2. Studies have revealed that the lower the SmO_2 values, greater the muscle oxygen extraction due to training load, but their interpretation is unknown in AWC activities. In this context SmO_2 measurement could provide important information similar to mVO_2, observed through the difference between SmO_2 at the exercise beginning divided by SmO_2 at the exercise end (ΔSmO_2), this is known as the amplitude method.[8] It also possible to measure the time in which the SmO_2 minimum values are reached as a performance and health factor.[6] Recently, the critical oxygenation (CO) model was published by Feldmann and Erlacher,[9] which represents "the greatest metabolic rate that results in wholly oxidative energy provision, and means that energy supply through substrate-level phosphorylation reaches a steady-state". The CO can be obtained through the SmO_2 values at the beginning and the end exercise.

Studies carried out by Bosquet in 2015 and 2016 reveal that a specific test of 30 contractions (approximately 38 seconds) at the peak torque isokinetic could be interpreted as AWC values.[10,11] But the peak torque is an indirect calculation of the anaerobic metabolism in the absence of laboratory instruments to measure energy pathways and oxygen consumption. So, an alternative to predict the AWC values could be the muscular oxygen consumption (mVO_2) with a linear regression analysis from the beginning to the end of the exercise (point to point), this is a methodology known as "slope"[8] and is accurate, however it is difficult to take to daily training due to the lack of clarity to interpret the data in a field of play environment. However, the CO is a new model much easier to interpret in the field, but its relationship to the AWC is unknown. We hypothesize that CO has the ability to explain the AWC performance in athletes due to its close relationship with critical power measured in previous studies.[9,12]. The aim of this study is report the relationship between CO and AWC during high intensity tests.

Methods

Participants

A total of twenty-two male players (age 22.5 ± 4.6 years, weight 89.8 ± 12.6 kg, height 176.4 ± 7.8 cm, sports participation 9.1 ± 3.6 years) from the rugby Portuguese first division participated in the present study by signing a written statement of informed consent. To avoid any residual fatigue induced by recent training, participants were asked to refrain from strenuous exercise 48 hours before the tests. In addition, the research protocol was approved by the Scientific and Ethical Committee of the University of Extremadura with Number of registration: 131/2018 and it was in accordance with the principles of the Declaration of Helsinki.

Measures

- High-intensity isokinetic fatigue test (FAT)
  The test was performed on a Biodex System-3 Isokinetic Dynamometer (Biodex, System 3, NY, USA), which consisted of a 5-minute warm-up by pedaling at 100 watts with a cadence of around 100 revolutions per minute (rpm) on a cyclo-ergometer (ergometrics 900, ergoline Germany). Thereafter, the participant was seated on the dynamometer seat, which was adjusted as previously described.[13] The range of motion was 100° (0° corresponding to a full active extension). Afterwards, they performed 30 consecutive maximal reciprocal concentric contractions at an angular velocity of 180°/s. The work performed during the entire range of motion of each repetition was computed using the device's software and summed to obtain the FAT total work, peak moments (PM) torque was obtained for maximum and average, were taken in Newton-meter (N.M.).

- Portable NIRS
  SmO_2, mVO_2 and CO values were measured using the portable NIRS sensor (Moxy. Fortiori Design LLC, Minnesota, USA). The device was placed in the vast lateral quadriceps between the greater trochanter and the lateral femoral epicondyle. For the analysis, data were recorded (visible only for researchers) using the raw SmO_2 and point to point Hbb and O_2Hb signal was treated with a soft spline filter to reduce noise created by movement[14] Matlab® software (The MathWorks, Inc., Massachusetts, United States) and SmO_2 was observed in real time with ANT + technology (GoldenCheetah version 3.4, U.S.)
- NIRS - Data Analysis
  The mVO_2 these values were compared with the values obtained from the linear regression of oxygenated hemoglobin (HbO) divided by deoxygenated hemoglobin (Hbb) that indicates the true mVO_2. The SmO_2 slope was obtained from the difference between the SmO_2 value at the start of exercise and the muscle oxygen extraction (SmO_2 minimum value) during the FAT test (Figure 1). additionally, an expert assessed the CO based on the model proposed Feldmann and Erlacher,[9] finally, the times in sec of each SmO_2 point (a, b and c in figure 1) were obtained as variables indicating a better oxidative performance.[15]
  The formulas for each variable are presented below:
  mVO_2 = HbO - Hbb constant
  SmO_2 slope = SmO_2 Start – SmO_2 minimum (from point (a) to point (b)).
  CO model = SmO_2 slope - SmO_2 maximum (point (c))
  CO time (s) = SmO_2 maximum time (s) – SmO_2 minimum time (s)

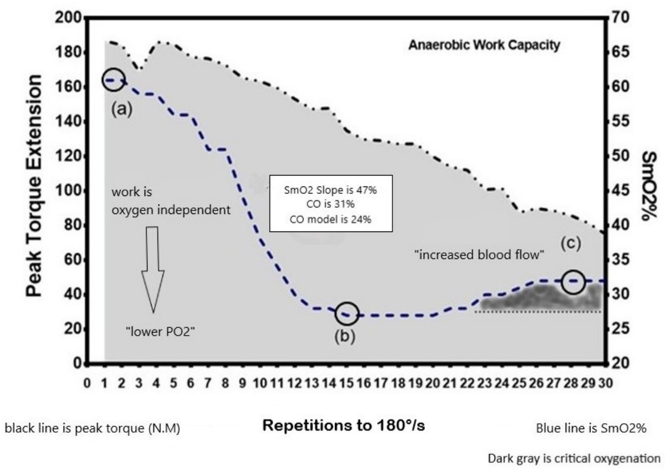

Figure 1. Dynamics of strength and SmO2% during a participant's evaluation in the dominant leg. (a)= Start of Exercise; (b)= Muscle oxygen extraction (Minimum SmO2); The SmO2 Slope is the difference between point (a) to point (b). Intramuscular oxygen Partial pressure (PO2). (c)= Critical Oxygenation (Maximum SmO2 after minimum SmO2).

Figure 2 shows how energy changes are obtained through the portable NIRS signal (MOXY), similar to the physiological mechanisms explained in the study by Conley et al.,[16] the review by Baker et al.[17] Sustainable AWC is determined at the muscular level, by the ability to maintain the ATP supply by decomposing PCr and by the products of glycolysis, which can inhibit the signal of oxidative phosphorylation, when exercise is very intense and therefore oxygen independent. This argument is based on magnetic resonance spectroscopy measurements of energy sources and sinks in vivo in human muscle and rattlesnake muscle during sustained contractions.[16] Likewise, the relationship between PO_2 and PCr can be explained by the balance maintained by creatine kinase, because a direct relationship was also found between the relationship [ATPI/ADPI] with PCr.[18] Sympathetic vasoconstriction is attenuated by higher PO_2 and blood flow, which is related to a higher oxidative rate that reduces lactate production.[19]

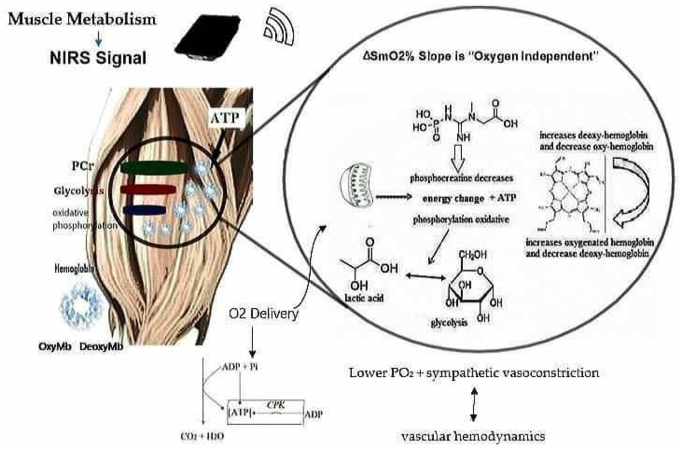

Figure 2. Interaction of hemoglobin in the muscle during "Anaerobic Work Capacity" exercise and the change of the energy pathways.

Statistical Analysis

SPSS software (IBM® SPSS® Statistics 22) was used to perform the statistical analysis. Data are presented as means and standard deviation (SD). Multiple linear regressions with Pearson's coefficients were also used to establish the respective relationships between NIRS parameters and AWC. The following criteria were adopted for interpreting the magnitude of correlation (r) between test measures: 0.1, trivial; 0.1–0.3, small; 0.3–0.5, moderate; 0.5–0.7, large; 0.7–0.9, very-large; and 0.9–1.0, almost perfect. Regression analysis is a statistical technique for determining the relationship between a single dependent (criterion) variable and one or more independent (predictor) variables. The analysis yields a predicted value for the criterion resulting from a linear combination of the predictors. Statistical significance was set up with p value <0.05.

Results

Table 1 shows the mean results obtained during the FAT test.

As expected, there was a high correlation between mVO_2 and SmO_2 slope (r= 0.96), and CO correlation with mVO_2 (0.85) and SmO2 slope (0.90). Furthermore, a greater amplitude of the SmO_2 slope would mean less anaerobic work (r = -0.58) and less force production. Also, a higher CO and CO time value is displayed with a lower AWC production (-0.63 and -0.76).

Table 1. Muscle oxygenation parameters obtained during the Anaerobic Work Capacity test.

ACW parameters	Mean ± SD
Peak Torque Max (N.m)	220 ± 30
Peak Torque Med (N.m)	170 ± 38
Total Work (Constant)	-3.01 ± 0.53
NIRS parameters	Mean ± SD
SmO_2 Start %	59.8 ± 7.4
SmO_2 minimum %	8.4 ± 14.1
SmO_2 medium %	21.1 ± 13.8
Critical Oxygenation %	20.8 ± 5.1
Critical Oxygenation % (seconds)	34 ± 8
SmO_2 Slope %	49.5 ± 14.9
mVO_2 (HbO/Hbb)	2.06 ± 0.53

Values are expressed as mean ± standard deviation.

Table 2 presents the multiple regression analysis models based on the SmO_2 dynamics. Moderate percentage correlations were observed in the equations to explain AWC by critical oxygenation. The use of time and weight are variables that explain the AWC by 64% and total work by 40%.

Table 2. Correlation between mVO2, SmO2 slope, CO and AWC during high-intensity isokinetic exercise.

Variables	AWC	mVO_2	SmO_2 Slope	CO	CO time
AWC	-	-	-	-	-
mVO_2	-0.60 0.021*	-	-	-	-
SmO_2 slope	-0.58 0.037*	0.96 0.00**	-	-	-
CO	-0.63 0.016*	0.86 0.00**	0.90 0.00**	-	-
CO time	-0.76 0.00**	0.22 3.06	0.52 0.032*	0.54 0.029*	-

**p value<0.05 and **p value <0.01 statistically significant. Qualitative interpretation of the correlation magnitude (r) between test measures: 0.1, trivial; 0.1–0.3, small; 0.3–0.5, moderate; 0.5–0.7, large; 0.7–0.9, very-large; and 0.9–1.0, almost perfect.*

Results are expressed in the procedure to calculate the AWC using the ΔSmO_2 data in athletes.

An example to evaluate AWC tests with the CO and CO time.

- *CO= SmO2 Slope – SmO2 maximun during the test (1)*
- *CO time= SmO2 maximum time – SmO2 minimum time (2)*
- *Weight= the subject's weight in kilograms*
- *AWC= 99.34 + 0.38 * CO (SmO_2%) – 2.40* CO time + 1.31* weight (kg) (3)*
- *Total Work= - 2.07 + 0.03* 1.02 * CO (SmO_2%) – 0.04* CO time + 0.06* weight (kg) (4)*

Example: a speed athlete weighting 64 kg runs a 400-meter test using a NIRS portable. The initial value is 65% (SmO_2 start) during the test and it is observed that the SmO_2 decreases to 26% (SmO_2 minimum) at the time of 26 seconds (SmO_2 minimum time). We would observe a SmO_2 Slope of 47%. Then a small increase and SmO_2 stabilization is observed at a value of 31% (SmO_2 maximum during the test). The test ended with a time of 48 seconds (SmO_2 maximum time).

From here we replace values with the formula:

CO= 24% of SmO2

CO time= 12 sec

AWC= 99.34 + (0.38 * 24) – (2.40* 12) + (1.31* 64 kg)

The AWC is 221 N.m

Answer: The athlete got an AWC of 221 N.m, presumably using the phosphocreatine energy system and glycolysis only in the first 36 sec. This procedure is using the CO model (Figure 3).

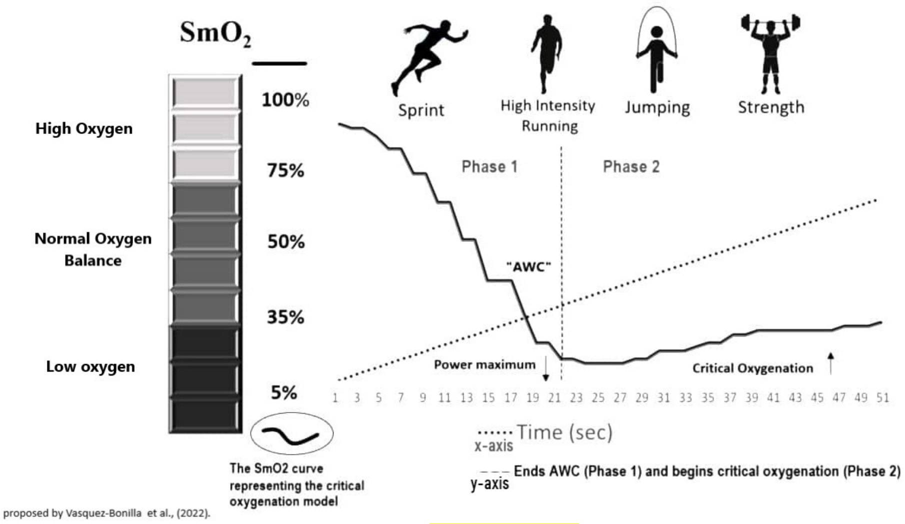

Figure 3. FALTA TÍTULO

Table 3. Multiple regression analysis models for mVO2 and AWC based on the measurement of SmO2 Slope and CO with portable NIRS.

Step prediction models and statistical analysis	Independent variable	Dependent variable	Non-standardized coefficient B	Standardized coefficient B	Prediction Percentage	F Value	Sig.
First order equation Linear regression	SmO_2 Slope	mVO_2	0.020 0.041	0.920	r= 0.92 r^2= 85%	203.194	0.000**
First order equation Linear regression	SmO_2 Slope	AWC (N.m)	4.871 -0.044	-0.570	r= 0.57 r^2= 32%	14.939	0.001*
First order equation Multiple linear regression	SmO_2 Slope Weight (kg)	AWC (N.m)	86.129 1.508 -0.243	0.504 -0.089	r= 0.60 r^2= 36%	7.955	0.033*
First order equation Multiple linear regression	Time weight (kg) SmO_2 Slope	Total Work (N.m)	1.834 0.075 0.017 -0.041	0.220 0.163 -0.425	r= 0.54 r^2= 29%	5.481	0.095
First order equation Multiple linear regression	Time weight (kg) SmO_2 Slope	AWC (N.m) when is "oxygen independent"	44.296 3.665 1.185 -0.562	0.377 0.396 -0.206	r= 0.65 r^2= 43%	4.504	0.016*
First order equation Multiple linear regression	CO CO time Weight (kg)	Total Work (N.m)	-2.070 0.031 -0.045 0.057	0.490 -0.262 0.49	r= 0.63 r^2= 40%	3.721	0.032*
First order equation Multiple linear regression	CO CO time Weight (kg)	AWC (N.m) when is "oxygen independent"	99.334 0.383 -2.394 1.312	0.250 -0.577 0.521	r= 0.80 r^2= 64%	10.058	0.017*

*p value<0.05 and **p value <0.01 statistically significant. Correlation coefficient R2 shows the explanatory model variance.

Discussion

This study shows how anaerobic work capacity and CO level are highly related to the development of power during the isokinetic test, which we presume is extracted in greater quantity from the energy of PCr and anaerobic glycolysis. This is a mechanism that can be measured by sports scientists and coaches during high intensity events.

There are many studies that support our results, the study by Denis et al.,[20] found a decrease in SmO_2 at the beginning of the isokinetic dynamometer extension test and later an increase due to a compensatory vasodilator effect to counteract the decrease in SmO_2. The explanation for this phenomenon is that at the beginning of exercise, an increase in intramuscular pressure occurs, restricts blood flow.[21] Vascular dilation and increased blood flow in the muscle is then promoted by increased production of metabolites (eg, K +, lactic acid). There is also an increase in muscle oxygenation detected by NIRS, which reflects the volume of blood in small vessels, including arterioles, capillaries and venules.[22] The temperature of the muscles and skin may have increased after the start of exercise, which also leads to dilation of the microvasculature.[22] This allows the acute positive regulation of vasodilator factors such as adenosine and nitric oxide (NO)[23] which respond to low local PO_2.[24,25] Low PO_2 values strongly affect the O_2-ATP reaction rate, but does not significantly compromise the use of intramuscular oxygen[26] and therefore does not substantially limit the consumption of oxygen. Rather deoxygenation under anaerobic conditions occurs by the transport of the myoglobin molecule, which also supports the diffusion-facilitating role that Mb can play in O_2 transport because Mb needs to be desaturated to facilitate the movement of O_2 from the blood to the cell.[26]

A lower intramuscular PO_2 also turns off phosphorus and affects the NIRS signal[27] which hinders myosin-actin bridge formation, and initiates the loss of contractile efficiency and force generation. Gomez-Carmona et al.,[28] have determined the oxygen differences as force production and found that, over time, they decrease. Generally, these differences in SmO_2 Slope are accompanied by the use of glycolysis to generate ATP, because arterial epinephrine levels stimulate glycolysis and are closely related to skeletal muscle lactate output.[18,29] Likewise, the Feldmann (2021) study, which used SmO_2 in a 3 min "all out" test, showed progress in finding the critical power, where a phenomenon similar to our study was observed: muscle oxygen extraction and CO, referring to the increased metabolic rate resulting in a fully oxidative energy supply.[9] It is the same model used in this study.

Likewise, energy metabolism can be explained by theoretical studies such as that by Gastin et al.[30] and Barclay[31] who argued that such a short test of anaerobic work reflects a decrease in performance, possibly due to the change in the metabolic pathway within the muscle. Similarly, other studies have indicated that a greater contribution of the energy-oxidative phosphorylation pathways is connected with an hyperaemia increase and blood flow, which causes the muscle to have a vasodilator effect and increases SmO_2.[5,32] In addition, during this metabolic process, the difference in the interindividual response of the subjects depends on the capacity of the cardiovascular system and the haemodynamics of the capillary beds to maintain the supply of oxygen in the muscle. This is observable in the almost complete desaturation in the anaerobic peak test which can occur due to relatively restricted blood flow.[4,27] Then, Increased blood flow is a mechanism that attenuates sympathetic vasoconstriction in active muscles by metabolic events in contracting skeletal muscle, in part by the activation of ATP-sensitive potassium (KATP) channels. Sympathetic vasoconstriction is mediated by the endogenous vasodilator NO, which is necessary to optimize muscle O_2 perfusion.[33,34] It is important to clarify that the greater activation capacity of type II fibres to extract oxygen through the glycolytic pathway is necessary to achieve better performance in high intensity areas and maintain a greater force and power production. because type II fibres need less oxygen to function.[32]

Many studies have observed a non-linear (hyperbolic) relationship of muscle oxygenation in high-intensity zones.[5,35] PO_2 is reduced and then remains constant despite a large continuous increase in work rate. Intracellular oxygenation does not, however, fall linearly with increasing metabolic rate, which indicates oxygen availability from submaximal exercise to single-leg knee extensor maximum.[29] Although the findings are straightforward, there are many far-reaching implications from these data. Changes in oxygenation levels over short periods have not been measurable with portable NIRS, and sometimes not with expensive instruments and laboratory tests. However, we can provide an explanation of this important phenomenon in measurements of oxidative metabolism at the local level, which could lead to better athletic performance. This may be a possible explanation for the lack of linearity. An important point is that improved muscle oxygenation during exercise leads to improved K+ regulation in humans.[36]

Regarding mVO_2 and SmO_2, during an exclusively anaerobic work test (sprint, jump, etc.), they report important information for the AWC assessment, however the objective of this study is to offer as an alternative analysis the "method of amplitude " which consists of taking the differences in the slope of SmO_2 up to the point at which it reaches the plateau[8]- that is, at best power production. Also provide sufficient information on the CO model recently published by feldmann[9](Figure 3).

It is worth noting that our study is an explanatory model with multiple regressions; to date there are no studies that only compare anaerobic work with SmO_2. In our study, we presumed that critical oxygenation could predict anaerobic work due to the similarity of the SmO_2 slopes and mVO_2 as variables identified in the analysis of muscle oxygen extraction;[37] however, that calculation is better when only the CO is used (Figure 3).

The main limitation of this study is that is does not measure the contribution of each energy pathway, as in the studies of Milioni et al.,[38] where a gas analyzer is needed, in addition to measurements of vascular dilation and muscle blood flow. However, we can overlap these data due to the high correlation of SmO_2 with VO_2 and blood flow.[39] This study proposes an advance in the physiology of muscle oxygenation that is applicable in a practical way by coaches in the field and by researchers to determine the performance of athletes and to plan training.

Summary, the critical oxygenation is associated with AWC test and is a peripheral performance factor. In addition, this information could be used as a new portable NIRS methodology in sports science studies and by coaches assessing muscle metabolism in maximum speed and power tests, sprint trials, jumps and intermittent work intervals to scientifically support the sports training. Processed data can be exported to NIRS instruments or integrated into smartphone apps and software. However, this information must be handled with care by scientists, as it must be tested and supported with other anaerobic tests to improve the AWC calculation.

Authotship. All the authors have intellectually contributed to the development of the study, assume responsibility for its content and also agree with the definitive version of the article. **Conflicts of interest.** The authors have no conflicts of interest to declare. **Funding.** This study has been supported by the Government of Extremadura with funding from the European Regional Development Fund under grant (Ref: GR18003), QUERCUS + 2018/19 (Erasmus + Internships). **Acknowledgements.** XXXXX. **Provenance and peer review.** Not commissioned; externally peer reviewed. **Ethical Responsabilities.** *Protection of individuals and animals:* The authors declare that the conducted procedures met the ethical standards of the responsible committee on human experimentation of the World Medical Association and the Declaration of Helsinki . *Confidentiality:* The authors are responsible for following the protocols established by their respective healthcare centers for accessing data from medical records for performing this type of publication in order to conduct research/ dissemination for the community. *Privacy:* The authors declare no patient data appear in this article.

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