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Acute Fatigue Impairs Heart Rate Variability and Resting Muscle Oxygen Consumption Kinetics

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Featured Application: Importantly, measurements obtained through VOT-NIRS can vary significantly post-exercise, which should be considered by researchers before application.

Abstract: This study evaluated the influence of acute fatigue on heart rate variability (HRV) and muscle oxygen saturation (SmO_2) at rest, as well as the reliability of SmO_2 data measured using near-infrared spectroscopy (NIRS) during a vascular occlusion test (VOT). Twelve physically active subjects participated. Measurements included perceived muscle soreness using the visual analog scale (VAS pain), HRV parameters, variables of resting SmO_2 (desaturation and resaturation), and reoxygenation kinetics (mean response time, MRT) through a VOT-NIRS located in the vastus lateralis (VL). Measurements were taken at three points: 24 h before, before exhaustive exercise, and 30 min after exhaustive exercise. The results indicated that acute fatigue increased resting muscle oxygen consumption in desaturation (+22 SmO_2) and resaturation (+18 SmO_2), improved MRT (−15 s), and elevated sympathetic nervous system (SNS) activity, as observed in the R-R interval (−262 ms) and SNS index (+0.5). HRV significantly influenced desaturation (r^2 = 0.69), resaturation (r^2 = 0.60), and MRT (r^2 = 0.54). Reliability was established with an ICC of 0.49 and 0.63 for desaturation and resaturation, respectively. Real changes in desaturation and resaturation should be considered $\geq$ 7% SmO_2 at rest and $\geq$ 11% SmO_2 to avoid daily fatigue interference. In conclusion, acute fatigue increases resting SmO_2 consumption and is associated with higher SNS activity and increased VAS pain.

Keywords: muscle oxygenation; vascular occlusion; near-infrared spectroscopy; muscle fatigue; sympathetic nervous system

1. Introduction

Currently, in sports sciences, sensors using non-invasive near-infrared spectroscopy (NIRS) technology are employed to measure muscle oxygen saturation (SmO_2), defined as the ratio of oxygenated hemoglobin (O_2Hb) to total hemoglobin (tHb) ($SmO_2 = O_2Hb \div tHb \times 100\%$) [1]. SmO_2 can be evaluated during exercise, and over the past decade, its assessment at rest has attracted researchers, allowing for the study of muscle metabolism through a vascular occlusion test (VOT) without requiring physical exertion [2,3]. A VOT shows how the muscle desaturates and resaturates after oxygen deprivation, indicating how muscle oxygen is utilized and recovered [4]. One of the primary objectives of a VOT is to identify "reactive hyperemia", a physiological phenomenon where blood flow temporarily increases following a brief interruption, which is useful for detecting endothelial capacity deficiencies [5]. A VOT-NIRS has demonstrated a slower oxygen recovery rate and a lower reactive hyperemia response in diseased populations compared to healthy subjects [6–9]. Additionally, the kinetics of muscle reoxygenation during a VOT describe the speed at which a muscle reoxygenates after ischemia and the restoration of blood flow, indicating non-invasively skeletal muscle oxidative capacity [10].

Recently, VOT protocols have been suggested as an important tool for determining sports performance [11,12] since they allow for the examination of muscle adaptations from a metabolic perspective [2]. However, few researchers use VOT-NIRS to identify performance improvements, partly due to the numerous methodologies and lack of consensus on data collection. It is important to highlight that the timing of VOT-NIRS measurements can cause variations in SmO_2 for athletes. For example, exercise-induced acute fatigue can destabilize muscle oxygen transport [13]. It has been described that female footballers do not return to stable SmO_2 levels after an official match [13], and faster reoxygenation kinetics of resting SmO_2 has been associated with severe injury risk compared to controls in footballers [14]. However, it was a short VOT (30 s) that allowed for the identification of adaptations to training [15]. Additionally, SmO_2 dynamics, such as desaturation and resaturation, have been related to the power of a countermovement jump (CMJ) before and 24 h post-exercise [12]. CMJ performance decreased 24 h after the match due to neuromuscular fatigue and was related to SmO_2 at rest [12,13].

SmO_2 measured with VOT-NIRS has the potential to track improvements in muscle oxidative capacity over prolonged training periods (i.e., six months) [2]. Most studies use VOT-NIRS at rest without prior exercise, which causes acute fatigue. Conversely, other studies have assessed post-exercise VOT-NIRS after a time trial and have identified a correlation with VO_2max [16]. Additionally, resting SmO_2 showed better predictive power for performance (92.7%) compared to other variables such as power, heart rate (HR), and VO_2max, which are metrics widely used in endurance sports [16]. However, when a VOT-NIRS was conducted on separate days without the influence of prior exercise, no relationship with VO_2max was found [17]. In contrast, Beever et al. [11] found a correlation between resting oxygen consumption and VO_2max in tests conducted on separate days without prior exercise influence, but this relationship was only observed in the vastus lateralis (VL) muscle and not in the gastrocnemius muscle, causing controversy among researchers. VOT-NIRS protocols have differences in application times, and researchers should consider this to be a possible interference in the results due to increased blood flow resulting from post-exercise or acute fatigue [18]. Based on the above, other studies have suggested a 30 min rest period to allow blood flow to return to resting conditions [19]. However, even after 30 min, the vasodilatory mechanisms from exercise may still be present, altering the VOT-NIRS results [20].

From a physiological perspective, oxygen transport during periods of acute fatigue is influenced by the response of the autonomic nervous system (ANS). This is due to a reduction in cardiac output associated with an elevated HR, leading to reduced heart rate variability (HRV), which measures fluctuations in the intervals between successive heartbeats (R-R intervals). High HRV after physical exercise indicates good autonomic balance and greater parasympathetic activity, while low HRV may suggest sympathetic dominance

or stress [21]. HRV measurement is more standardized; it is typically measured at rest and under controlled conditions to avoid interference from exercise-induced fatigue [22]. Conversely, it is also used to identify acute exercise-induced fatigue, making it a practical variable for daily fatigue assessment [21,23]. Using HRV and resting SmO_2 with VOT-NIRS could confirm the influence of acute fatigue from the ANS and clarify the controversy in performance evaluation results.

The current literature shows that some authors often need to pay more attention to the importance of timing in SmO_2 measurements, whether pre- or post-exercise, leading to potential confusion in performance prediction studies [16]. The timing of VOT-NIRS measurements should be clearly defined according to the research objective to accurately identify the measurement point and avoid discrepancies in results. Therefore, the present study aims to (1) investigate the influence of HRV on muscle oxygenation to examine the potential interference of exhausting physical exercise over VOT-NIRS and SNA measurements, and (2) to evaluate the reliability of resting oxygenation measurements between days and with the influence of post-exercise fatigue. We hypothesize that post-exercise SmO_2 changes will affect data interpretation by researchers and that HRV may influence the resting SmO_2 response.

2. Materials and Methods

2.1. Participants

A total of 12 physically active subjects (years 24.3 ± 4.4 old, weight 72.3 ± 7.8 kg, height 176.1 ± 4.1 cm, and fat 10.4 ± 2.2%) participated in the study. The participants engaged in regular physical activity based on the criteria of more than one hour per session and more than five days per week and were recruited from a strength training gym. The inclusion criteria were the absence of diseases and/or regular use of medications that affect the cardiovascular system and/or interfere with the ANS. The evaluation and intervention protocols were approved by the Scientific Ethics Committee of the Universidad Viña del Mar (Code R62-19a) and adhered to the standards and principles of the Declaration of Helsinki. All participants were informed of the objectives and risks of the protocol and subsequently signed written consent before data collection.

2.2. Study Design

This was a quantitative, cross-sectional study design. Initially, participants were instructed on the measurement protocol, which included HRV and SmO_2 at rest by VOT. Measurements were taken at three points (Figure 1): the first measurement was conducted 24 h before exercise (point 1) to establish baseline values, the second measurement was taken 30 min before exercise (point 2) to assess the reliability of the tests without fatigue, and the third measurement was performed 30 min after the exercise (point 3). A strenuous plyometric jump exercise was conducted to induce muscle fatigue before the measurement at point 3. The study was conducted in a controlled-temperature sports physiology laboratory (21–2 °C). Each participant was assessed in the morning, between 9:00 a.m. and 11:00 a.m., to avoid circadian rhythm disturbances. Additionally, participants were instructed to refrain from any strenuous physical activity 48 h before the first measurement and to avoid caffeine consumption for 2 days before testing.

Measurements

Induction of localized muscle damage

Participants completed 10 × 10 repetitions of plyometric jumps from a 0.6 m box (10 s between jumps, 1 min between sets). Volunteers were instructed to leave the box with one foot, land with both feet together, and aim for a knee angle of approximately 90° before performing a maximum vertical jump, although jump height was not recorded. The plyometric exercise was monitored by a strength and conditioning expert. This protocol is an appropriate method to induce muscle damage, as used in several previous studies [24].

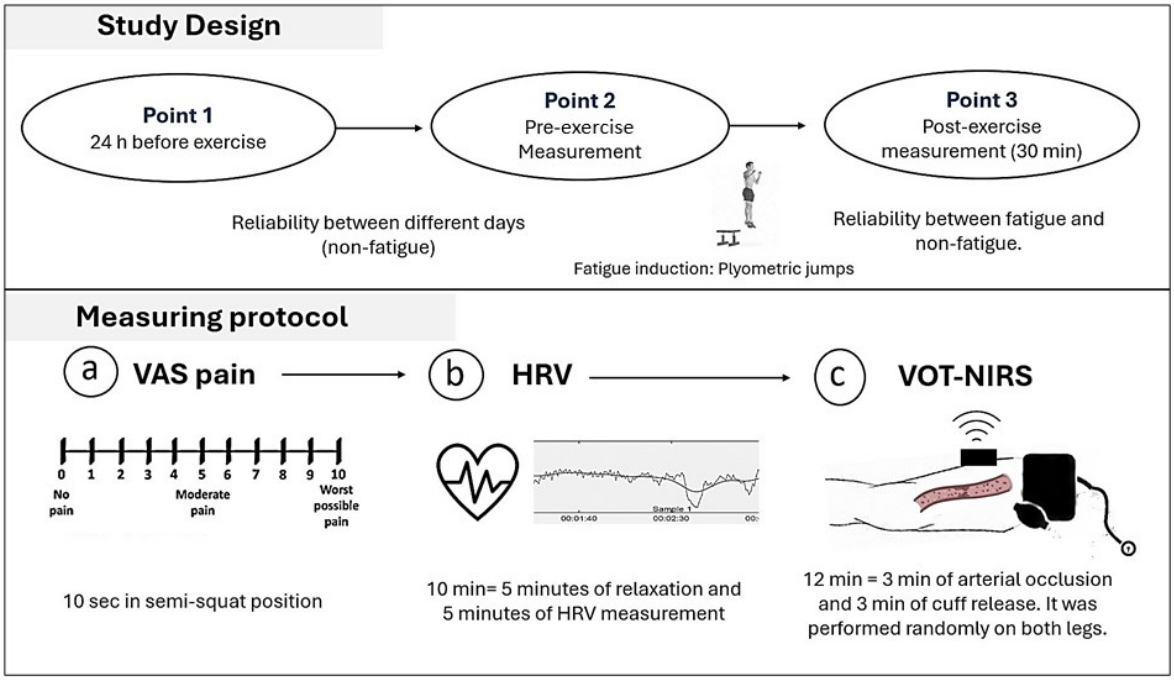

Figure 1. Protocol design. **Note.** (**a**) VAS pain = Visual Analog Scale; (**b**) HRV = Heart Rate Variability; (**c**) VOT-NIRS = Vascular Occlusion Test using Near-Infrared Spectroscopy. Protocol of fatigue induction 10 × 10 repetitions of plyometric jumps from a 0.6 m box (10 s between jumps, 1 min between sets). The measurement protocol was carried out at the three measurement points.

Perceived muscle soreness

Each participant was asked to rate their perceived muscle pain in the thighs using a scale from 0 to 10. The scale provided participant responses on a continuum anchored by cues written as zero, indicating no muscle pain, and ten, indicating muscle pain. It indicated that the muscles were too sore to move. With their hands on their hips, participants were instructed to bend their knees while keeping both heels in contact with the floor throughout the test. Participants were then asked to indicate their level of perceived pain according to the rating scale, reflecting the location of the muscle pain on the continuum. This technique has been successfully used in previous studies [25,26].

Heart Rate Variability (HRV)

After a 5 min rest period in a supine position, HR was recorded beat-by-beat for 5 min, with respiratory rate controlled at 15 breaths per minute (i.e., 2 s of inhalation and 2 s of exhalation) [27]. A Garmin HRM-PRO chest strap heart rate monitor (Garmin Ltd., Olathe, KS, USA) was used. The R-R interval data were sent to a WIMU PRO device and exported as a CSV file. This file was then processed using Kubios HRV software (Kubios HRV Standard, ver. 3.0.2., Kubios, Kuopio, Finland), where time-domain and frequency-domain variables were analyzed [28,29]. The software's algorithm automatically corrected for ectopic beats by interpolating adjacent R-R intervals using a cubic spline interpolation method with low filtering power [29]. This low filter option was selected because previous studies have suggested that it sufficiently removes measurement errors while preserving inherent R-R interval variability [30]. Raw R-R interval data were used to derive indices for the parasympathetic nervous system (PNS), the sympathetic nervous system (SNS), the stress index, the mean R-R interval (mean RR), the standard deviation of the R-R intervals (SDNN), and root mean square successive differences of R-R intervals (RMSSD). The PNS was calculated based on mean RR, RMSSD, and SD1, while the SNS was calculated based on mean heart rate, Baevsky's stress index, and SD2 [31,32].

Near-infrared spectroscopy (NIRS)

SmO_2 measurements were obtained using a NIRS sensor (Moxy, Fortiori Design LLC, Minnesota MN, USA), which was placed on the vastus lateralis of the non-dominant leg, 15 cm above the superior border of the patella and 5 cm lateral to the midline of the thigh, aligned with the muscle fibers. The Moxy sensor was set to a sampling frequency of 10 Hz. Data were transmitted via ANT+ technology to Golden Cheetah software (GoldenCheetah version 3.6. CO, USA), allowing real-time visualization for the researchers. The NIRS device was secured with medical adhesive tape, and during measurements, the limb was covered with a black cloth to prevent interference from ambient light. Skinfold was assessed with a skinfold caliper (Harpenden®®, London, UK). The skinfold thickness was less than half the distance between the sensor's emitter and detector (~2.5 cm). The average skinfold thickness was 11.4 ± 2.3 mm, which was below the distance between the NIRS light emitter and detector. Measurements were performed by an experienced researcher in skinfold measurements. The resting SmO_2 measurement was conducted by an experienced investigator using the occlusion technique.

Vascular Occlusions Test (VOT)

To assess the dynamics and kinetics of SmO_2 in the VL muscle, a VOT protocol was used involving a 220–250 mmHg occlusion for 3 min [33]. Before the VOT, a 1 min baseline measurement of SmO_2 was recorded. Following this, a manual pressure cuff (NIBP Cuff for PM900, London UK) was inflated on the distal thigh to induce ischemia for 3 min, causing visible muscle desaturation (Figure 2). After the 3 min occlusion, the cuff was released, and 3 min of reperfusion was allowed to analyze muscle resaturation and reactive hyperemia through changes in SmO_2 [3]. Subsequently, the participant rested for another 2 min (totaling 5 min) before repeating the VOT measurement on the other leg. VOT measurements were performed randomly on either the dominant or non-dominant leg to allow recovery of blood flow from ischemia and to avoid interference from blood flow redistribution [4]. For data analysis, the average of the two measurements from each leg was used.

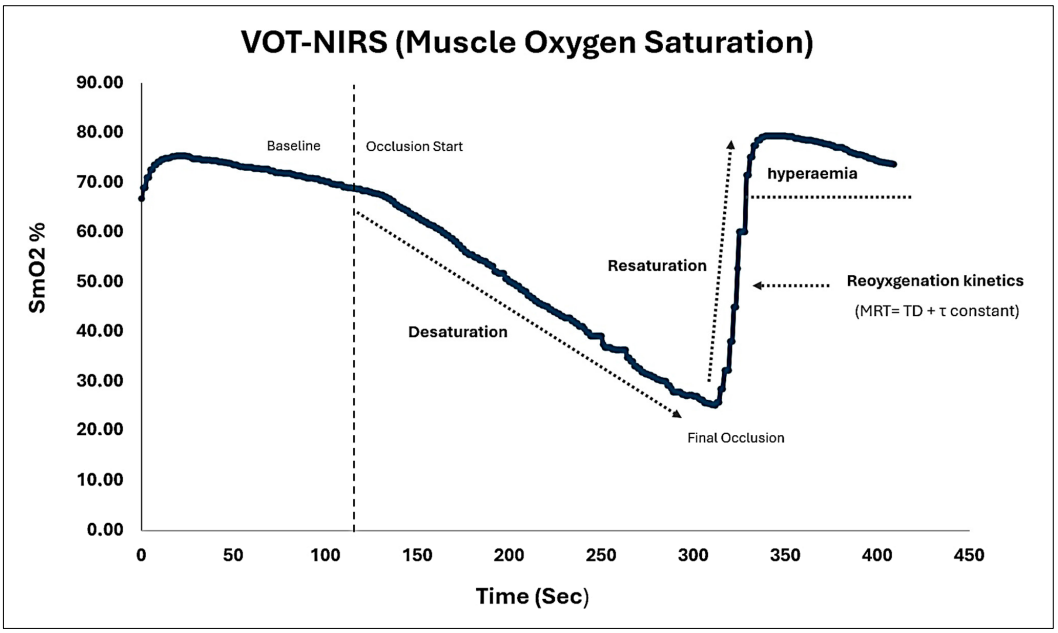

Figure 2. Visualization of vascular occlusion test. **Note.** Baseline = Average values of the 30 s before starting VOT-NIRS; Desaturation = SmO_2 decrease during the ischemic period (3 min); Resaturation = SmO_2 increase during the recovery period; Reactive hyperemia = SmO_2 increase compared to baseline values. Reoxygenation Kinetics = Oxygen utilization rate based on mean response time (MRT), which is equal to the sum of the delay time (DT) and the time constant (Tau).

Resting Muscle Oxygen Consumption Data

(a). SmO_2 Dynamics: The analysis of SmO_2 dynamics involved examining the following curves: desaturation (the difference between SmO_2 at the beginning and end of occlusion), resaturation (the difference between SmO_2 stabilized at the end of recovery and SmO_2 at the end of occlusion), and reactive hyperemia (the area under the SmO_2 curve from the end of recovery to the beginning of occlusion) [6,13–15] (See Figure 2);

(b). Reoyxgenation kinetics: The post-occlusion reoxygenation kinetics were derived from oxyhemoglobin [oxy (Hb+Mb)] values taken at 1 s intervals and plotted against time [17,34,35]. The curve was fitted to an exponential model using the least squares method to describe the oxyhemoglobin response following arterial occlusion. The equation used was as follows:

$$Y(t) = Y0 + A \times (1 - e^{-(t-TD)/\tau})$$

where Y0 represents the baseline value (i.e., the last 20 s before the end of the 3 min arterial occlusion), A is the amplitude of the oxy[Hb+Mb] response, TD is the time delay immediately after the occlusion is released, and τ (tau) is the time constant associated with the slope recovery.

This fitting was performed using a combined filtering and correlation method [36,37]. The point at which the correlation reached its maximum was considered the starting point of the mono-exponential curve, while the endpoint of the mono-exponential curve was determined by identifying the highest value. Finally, the mean response time (MRT) was calculated as the sum of TD and τ constant. The goodness of fit (R^2; coefficient of determination) was deemed satisfactory if it exceeded 0.85 [36]. All curve fitting and reoxygenation kinetics data analysis was performed using SigmaPlot 10 and SigmaStat 3.5 software (Systat Software Inc., San Jose, CA, USA).

Statistical Analysis

JAMOVI®® version 2.3.21 for Windows (Sydney, Australia) was used for statistical analysis. The variables were presented with means and standard deviations to describe muscle oxygenation, HRV data, and VAS pain at the three measurements: Points (1 and 2) without fatigue and point 3 measurements with fatigue. First, a normality test was performed through the Shapiro–Wilk test. Then, an analysis of variance (ANOVA) was applied to establish differences between conditions; a post hoc was performed with the Bonferroni test. A partial eta squared test (η^2_p) was also performed considering values of <0.01, between 0.01 and 0.059, and >0.138, classifying as small, medium, and large effect sizes, respectively [38]. The variables that showed a significant difference were then used in a multiple linear regression analysis to identify the influence of HRV (independent variable) on muscle oxygen consumption variables (dependent variables). The condition (without fatigue or with fatigue) was included as a factor that modulates the multiple regression. The regression model was visualized using a residual analysis plot to identify outliers. Additionally, a Pearson correlation analysis was applied between VAS pain and muscle oxygen consumption variables. A p-value of <0.05 was considered statistically significant in all tests. The reliability analysis included the calculation of the typical error of the estimate (TEE) (change in SD $\div \sqrt{2}$), which represents the noise of the signal for each variable. Furthermore, we analyzed the intraclass correlation coefficient (ICC; 1). Values below 0.5 indicate poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values above 0.9 indicate excellent reliability [39]. The coefficient of variation percentage (CV) was also estimated based on the TEE [(SD $\div$ mean) $\times$ 100], and the Minimum Detectable Change (MDC) was calculated as $1.96 \times TEE \times \sqrt{2}$ [40].

3. Results

Table 1 presents the muscle oxygenation data for the three measurement points. The post hoc analysis showed significant differences in the final occlusion between points 1 and 2 compared to point 3 ($p = 0.002$). Differences were also found in desaturation between point 1 ($p < 0.001$) and point 2 ($p < 0.001$) compared to point 3. The resaturation differences were observed between point 1 ($p < 0.001$) and point 2 ($p < 0.001$) compared to point 3. Concerning reoxygenation kinetics, differences were found at baseline (Y0) between point 1 and point 3 ($p = 0.042$). In amplitude, differences were observed between point 1 ($p < 0.001$) and point 2 ($p < 0.001$) compared to point 3. The Tau was lower at point 3 compared to point 1 ($p = 0.030$) and point 2 ($p = 0.004$). Similarly, MRT was longer at point 1 ($p = 0.030$) and point 2 ($p = 0.004$) compared to point 3.

Table 1. Assessment of resting muscle oxygen consumption during vascular occlusion test under fatigue and non-fatigue conditions.

	Descriptives	Point 1		Point 2		Point 3		F	*p*	η^2p
		Mean	SD	Mean	SD	Mean	SD			
Occlusion variables	Initial Occlusion	70.5	6.9	72.8	9.2	77.5	5.0	1.88	0.177	0.152
	Final Occlusion	31.7	9.3	32.3	8.1	17.4	7.2	8.26	0.002	0.440
	Recovery Occlusion	77.4	5.2	78.1	8.2	81.6	3.7	1.11	0.348	0.096
SmO_2 dynamics	Desaturation	38.8	6.2	40.5	6.2	60.0	8.4	22.4	<0.001	0.681
	Resaturation	45.7	7.3	45.8	8.4	64.1	9.5	12.5	<0.001	0.544
	Hyperemia	6.9	5.9	5.3	5.0	4.1	4.4	0.554	0.583	0.050
Reoxygenation kinetics	Baseline (Y0)	6.8	1.4	6.4	1.1	5.2	0.6	3.99	0.034	0.275
	Amplitude	3.4	0.7	3.5	1.1	6.0	0.8	22.3	<0.001	0.680
	Time Delay	2.7	0.6	2.6	0.6	4.8	0.6	0.516	0.604	0.047
	Tau	35.9	14.5	41.3	13.5	19.0	5.4	7.58	0.003	0.419
	MRT	37.4	10.3	32.6	15.2	21.8	8.2	7.60	0.033	0.420

Note. *p*-value < 0.05 statistically significant. Effects size: Small ≤ 0.01, medium ≥ 0.059, and large: >0.138. Point 1 = one day before without fatigue; point 2 = without fatigue, and point 3 = with fatigue conditions. Tau = Constant time and MRT = Mean Response Time.

Table 2 shows that participants in point 3 presented lower interval R-R ($p = 0.025$), higher Mean HR ($p = 0.013$), and higher SNS Index values ($p = 0.033$). For the other variables, no differences were observed between conditions. VAS pain showed differences between point 1 ($p < 0.001$) and point 2 ($p < 0.001$) compared to point 3.

Table 2. Heart rate variability and perceived muscle pain under fatigue and non-fatigue conditions.

Descriptives	Point 1		Point 2		Point 3		F	*p*	η^2p
	Mean	SD	Mean	SD	Mean	SD			
Interval R-R	1113.7	192.3	1070.3	235.0	852.3	117.7	4.44	0.025	0.297
RMSSD	258.6	271.7	196.2	288.3	79.8	50.1	1.24	0.310	0.106
SDNN	324.7	426.0	155.1	199.6	71.9	29.2	1.79	0.191	0.146
PNS Index	6.7	8.0	5.3	9.9	0.8	1.4	1.42	0.264	0.119
SNS Index	−1.6	0.9	−1.1	1.5	−0.1	0.7	4.03	0.033	0.277
Mean HR	55.2	9.4	58.8	11.9	71.6	10.0	5.34	0.013	0.337
VAS pain	0.50	0.75	1.25	1.03	6.87	0.83	125	<0.001	0.922

Note. *p*-value < 0.05 statistically significant. Effects size: Small ≤ 0.01, medium ≥ 0.059, and large: >0.138. Point 1 = one day before without fatigue; point 2 = without fatigue, and point 3 = with fatigue conditions. PNS = Parasympathetic nervous system, SNS = sympathetic nervous system, SDNN = the standard deviation of the R-R intervals, and RMSSD = Root mean square successive differences of R-R intervals.

Figure 3 presents probability plots based on the percentage of prediction and standardized residuals. The regression model included the following variables: (a) desaturation,

(b) resaturation, (c) hyperemia, and (d) MRT. The HRV variables were R-R interval, SNS index, and average HR, as these showed the most significant changes.

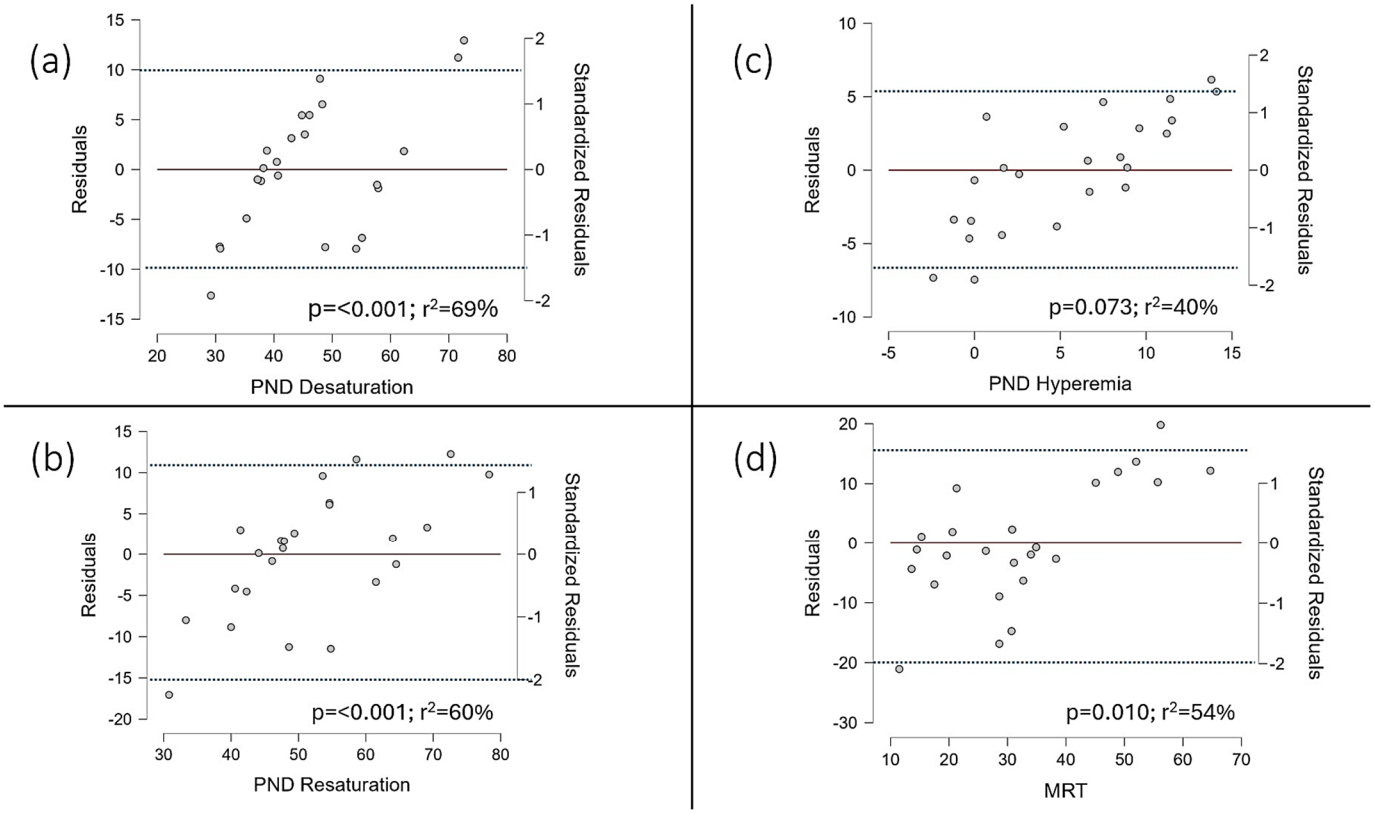

Figure 3. Multiple linear regressions between heart rate variability and resting muscle oxygen consumption. **Note.** The regression model included the following HRV residual analysis as an independent variable and dependent variable: (**a**) desaturation, (**b**) resaturation, (**c**) hyperemia, and (**d**) MRT. The condition (without fatigue or with fatigue) was included as a factor that modulates the multiple regression.

The data fit the multiple regression model reasonably well for the desaturation response (residual = 0.00 ± 6.57; predictive values = 46.44 ± 9.91 SmO_2) with outliers at ≥30% and ≤70% SmO_2. For the resaturation response (residual = 0.00 ± 7.60; predictive values = 51.90 ± 9.29 SmO_2) with outliers at ≥30% and ≤70% SmO_2. For the hyperemic response (residual = 0.00 ± 3.91; predictive values = 5.46 ± 3.45 SmO_2) with outliers at ≥−0.3% and ≤15% SmO_2. For the MRT response (residual = 0.00 ± 10.07; predictive values = 32.18 ± 11.03 s) with outliers at ≥10 s and ≤60 s.

The statistical values from the multiple linear regression showed that HRV influenced the desaturation response (r^2 = 0.695; F = 8.205; $p < 0.000$), with the fatigue condition being significant (t = 4.763; $p < 0.000$). HRV also influenced the resaturation response (r^2 = 0.599; F = 5.384; $p = 0.003$), with the fatigue condition being significant (t = 4.386; $p < 0.000$). However, HRV did not show significance for hyperemia (r^2 = 0.408; F = 2.481; $p = 0.070$), and the fatigue condition had no influence on hyperemia (t = 0.532; $p = 0.601$). Finally, HRV influenced the reoxygenation kinetics (MRT) response (r^2 = 0.545; F = 4.318; $p = 0.009$), with the fatigue condition being significant (t = −3.691; $p = 0.002$).

Figure 4 illustrates the correlation analysis between VAS pain and the resting muscle oxygen consumption obtained from VOT-NIRS. A significant correlation was observed between VAS pain and desaturation (r = 0.81, 95% CI = 0.60 to 0.91; $p \leq 0.001$), resaturation (r = 0.69, 95% CI = 0.39 to 0.85; $p \leq 0.001$), and MRT (r = −0.58, 95% CI = −0.80 to −0.23;

p = 0.003). However, no significant correlation was found between VAS pain and hyperemia (r = −0.264, 95% CI = −0.60 to 0.16; p = 0.212).

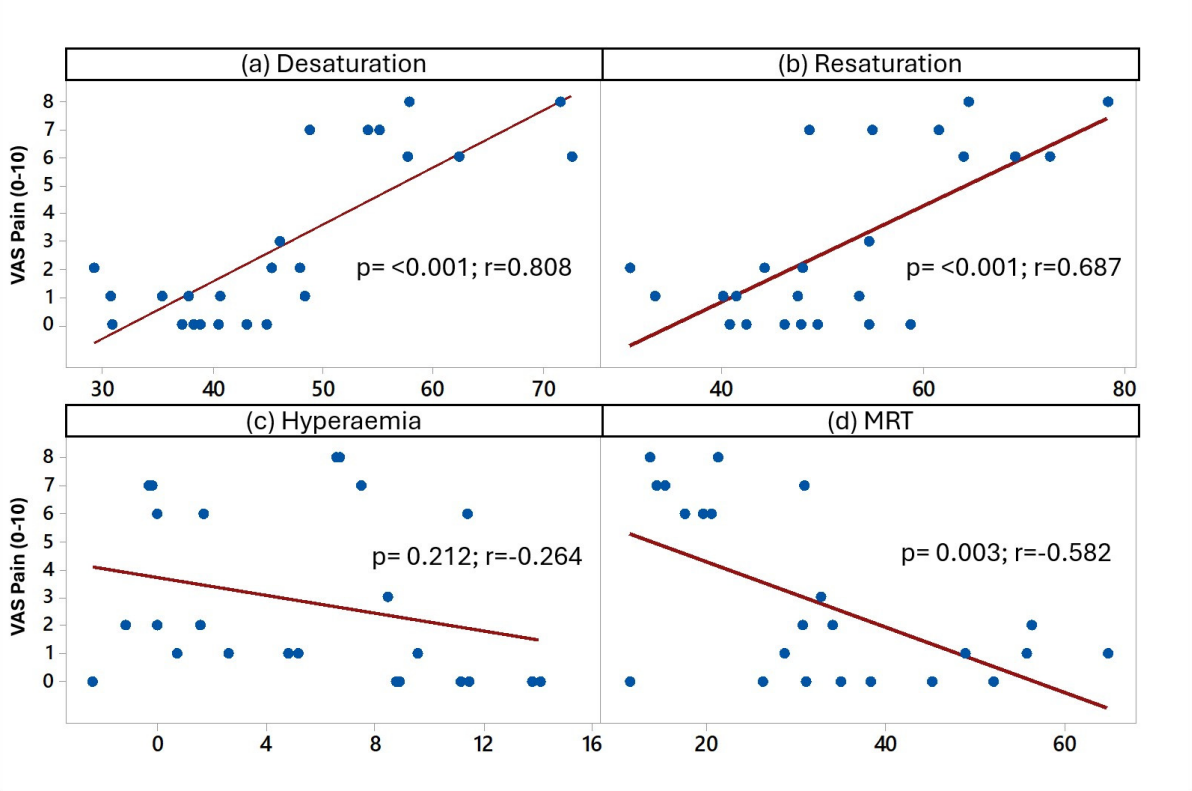

Figure 4. Correlation between perceived muscle pain and resting muscle oxygen consumption measured with VOT-NIRS. **Note.** The correlation analysis is shown in the graphs: (**a**) desaturation, (**b**) resaturation, (**c**) hyperemia, and (**d**) MRT.

Regarding reliability data between days and in the absence of fatigue, a moderate ICC was observed for resaturation (r = 0.63), hyperemia (r = 0.57), and MRT (r = 0.50). The CV was lower for desaturation (%CV = 5.97) and MRT (%CV = 9.50). The TE for desaturation and resaturation ranged from 5% to 11% SmO_2, with an MDC greater than 7% SmO_2. The TE for hyperemia ranged from 2% to 6% SmO_2, with an MDC of 4.3% SmO_2. For Tau and MRT, TE and MDC values are approximately 8.2 s and 13.7 s, respectively. For HRV measures, excellent ICC values were found for the R-R interval (r = 0.94), PNS index (r = 0.98), SNS index (r = 0.97), RSMMD (r = 0.98), and Mean HR (r = 0.91). However, the SDNN showed lower reliability with an ICC of 0.43. The CV was lower for the R-R interval (%CV = 2.95), RSMMD (%CV = 1.76), and SDNN (%CV = 1.78).

When comparing conditions without fatigue vs. with fatigue, moderate ICC values were found for the SNS index (r = 0.61) and Mean HR (r = 0.59). The variables with the least variability were desaturation (%CV = 2.0), resaturation (%CV = 2.6), and MRT (%CV = 2.8). For HRV, all variables showed the following CV: R-R interval (%CV = 1.0), PNS index (%CV = 9.3), SNS index (%CV = 9.6), RSMMD (%CV = 2.0), SDNN (%CV = 2.3), and Mean HR (%CV = 3.8). Overall, fatigue influenced desaturation and resaturation TE values, which were less than 10% SmO_2, with an MDC considered to be above 11% SmO_2. For hyperemia, TE was less than 5% SmO_2 with an MDC greater than 4% SmO_2, and the results were not significant. TE for MRT and Tau was around 12 s under fatigue conditions, with an MDC considered to be above 14.5 s. However, it is important to consider the confidence intervals, as data may fall within these ranges and should be consistent across measurements.

4. Discussion

The main finding is that acute fatigue increases resting muscle oxygen consumption and is associated with increased sympathetic nervous system (SNS) activity. Therefore, previous exercise can affect resting muscle oxygenation data measured by VOT-NIRS.

Resting muscle oxygen consumption increased under acute fatigue conditions, showing greater desaturation (−20.5%) and resaturation (+19.5%). This aligns with Bonilla et al. (2020), who found that acute fatigue increased SmO_2 slopes following an official soccer match and persisted for 24 h, correlating with elevated lactate dehydrogenase (LDH) levels. This increase is primarily due to Excess Post-Exercise Oxygen Consumption (EPOC) [41]. The rapid phase of EPOC occurs immediately post-exercise and can last up to 3 h [42]. A SmO_2 increase and an accelerated slope pattern (tau, MRT, desaturation, and resaturation) at rest are necessary to restore muscle homeostasis. This process aids in eliminating accumulated CO_2, rapidly redistributing blood flow to the vastus lateralis muscles post-exercise, and increasing thermogenesis to restore ATP and phosphocreatine levels. Furthermore, increased eccentric work from jumps raises the total energy cost covered by oxidative and glycolytic pathways in the muscle post-exercise [43].

Also, in conditions of muscle damage, the physiological mechanisms explaining the interaction between HRV and SmO_2 at rest have been studied [13,44,45]. The reduction in HRV is associated at the cellular level with increased levels of Creatine Phosphokinase (CPK) and reactive oxygen species (ROS) as byproducts of mitochondrial metabolism [46,47]. Additionally, there is an increase in peripheral vasodilation resulting from the activation of H1 and H2 histamine receptors. When muscle oxygen consumption increases at rest due to greater metabolic demand or fatigue, as measured by NIRS, vasodilatory substances such as prostaglandins, adenosine, and nitric oxide are present in ischemic tissue and are modulated by sympathetic vascular tone controlled by the autonomic nervous system (ANS) [48]. The post-exercise increase in SmO_2 depends on the training status, which is related to endothelial capacity, likely attributed to the release of endothelial-derived factors such as nitric oxide (NO), which possesses antiproliferative, anti-inflammatory, and antithrombotic properties and promotes vasodilation [49]. This interaction between the increase in HRV and oxygen consumption reflects a complex adaptive response in which the muscle seeks to enhance its ability to manage metabolic and oxidative stress while optimizing recovery after exercise. Although these mechanisms have not been exhaustively studied, they are related to increased SmO_2 at rest and mitochondrial capacity.

The hyperemic response showed a reduction under fatigue conditions (−2.8%), but this was not significant. This response indicates greater endothelial capacity, which was not evident in our study. Hyperemia responds immediately to exercise and returns to baseline within minutes; its stabilization speed depends on muscle oxygen consumption and the vasodilatory response [50]. Acute fatigue showed lower values 30 min post-exercise, with hyperemic response stabilizing faster due to increased oxygen transport speed [51]. These results align with the finding that reoxygenation kinetics were affected by acute fatigue, resulting in faster tau constant values [16]. Reoxygenation kinetics indirectly indicate mitochondrial function, which can significantly improve oxygen utilization and mitochondrial respiration, facilitating muscle homeostasis recovery [52]. Conversely, slower mitochondrial respiration is associated with poorer fitness [53]. Rapid reoxygenation may also aid in converting lactate to pyruvate, enhancing muscle recovery [54].

In the multiple regression analysis (Figure 3), increased muscle oxygen consumption was associated with elevated sympathetic activity, as evidenced by decreased R-R interval and increased SNS index and mean HR. HRV variables influenced desaturation ($r^2 = 0.69$) and resaturation ($r^2 = 0.60$). While no studies have combined VOT-NIRS with HRV, it is known that localized muscle fatigue after specific exercises increases blood flow oscillations and muscle oxygen consumption [55]. Tan et al. (2021) measured SmO_2 and tHb using spectral analysis, showing that muscle oxygen increases depending on the duration and intensity of previous exercise [56]. Sustained sympathetic nervous system activation in response to muscle metabolic demands can elevate blood pressure, heart rate, and

sympathetic vasoconstrictor activity [57], especially when muscle blood flow demand exceeds resting levels [58]. Increased metabolic demand and the need to maintain adequate oxygen and nutrient supply to muscles activate the sympathetic nervous system, leading to increased blood flow and decreased R-R interval, supporting recovery, effective circulation, and metabolite clearance post-exercise [59]. VOT-NIRS data is considered valid after ischemia with desaturation and resaturation values between $\geq$30% and $\leq$70% SmO_2, similar to other studies showing 40–58% in desaturation and resaturation slopes [8]. MRT responses between $\leq$10 s and $\geq$60 s are also consistent with Batterson's findings [16], which reported values from 20 s (fastest) to 50 s (slowest), suggesting comparable time constant measurements despite methodological differences in VOT-NIRS.

Increased resting muscle oxygen consumption was associated with VAS pain (Figure 4) during desaturation (r = 0.81), resaturation (r = 0.69), and MRT (r = −0.58). This aligns with Bonilla et al. (2020), who found a higher VAS pain correlating with increased resting SmO_2 (r = 0.65), with similar VAS pain values under acute fatigue conditions (6.1 and 6.8, respectively). While higher resting SmO_2 is debated, physiologically it might prevent increased muscle activation during exercise due to reduced vasoconstriction capacity, as more blood flow suggests enhanced metabolite clearance, which is known to cause pain [60]. Additionally, rapid oxygen consumption in acute measures could indicate pathology and potential lower limb injuries [14], all related to perceived muscle pain.

Regarding the reliability data found in this study between different days and without fatigue, moderate ICC was found for resaturation, hyperemia, and MRT. These results contrast with previous studies that reported baseline SmO_2 measurements with ICCs between 0.90 and 0.99 [13,14,19] and SmO_2 slopes ICCs from 0.77 to 0.97 [13,14,61]. However, these measures were taken in the gastrocnemius muscle, which has less error than the vastus lateralis. The vastus lateralis showed ICCs ranging from 0.35 to 0.91 [62]. NIRS-derived SmO_2 slope of resaturation has good reliability over various occlusion durations, with the highest reliability during longer occlusions of 3 to 5 min compared to 30 s and 1 min [63]. Additionally, a 3.4% SmO_2 error with an MDC of 9% SmO_2 was found, similar to our study with an MDC > 7% SmO_2 [62]. Although no studies have evaluated reliability under acute fatigue conditions, our results align with studies using VOT-NIRS, where desaturation and resaturation data range between 30% and 70% and an MDC > 11% of SmO_2. Hyperemia had a high CV of 40%, possibly contributing to the high variability and errors exceeding the reported mean values of TE = 6% SmO_2. HRV data showed higher ICCs of 0.91 to 0.99 and low CV variations of 1.8% to 2.9% compared to all SmO_2 variables. Overall, fatigue reduced data reliability (see Table 3), probably due to increased muscle oxygen consumption, supporting this study's hypothesis.

Table 3. Description of the reliability between the days and conditions of fatigue obtained from resting muscle oxygen consumption and HRV data.

	Reliability between Different Days (Non-Fatigue)				Reliability between Fatigue and Non-Fatigue Measurements			
Variables	**TE**	**ICC**	**CV%**	**MDC**	**TE**	**ICC**	**CV%**	**MDC**
Desaturation	7.3 (5.4 to 11.3)	0.49	5.97	7.4	8.5 (6.3 to 13.2)	0.04	1.96	8.6
Resaturation	7.4 (5.5 to 11.4)	0.63	14.4	7.5	9.4 (6.6 to 15.9)	0.18	2.63	11.1
Hyperemia	3.6 (2.6 to 6.1)	0.57	40.5	4.3	3.3 (2.3 to 5.6)	0.49	11.41	3.9
Tau	11.6 (8.2 to 19.7)	0.32	94.7	13.7	12.0 (8.5 to 20.4)	0.03	13.67	14.2
MRT	8.2 (5.8 to 13.9)	0.50	9.50	9.7	12.2 (8.7 to 20.8)	0.45	2.80	14.5
Interval R-R	51.53 (36.5 to 87.5)	0.94	2.95	60.9	141.7 (100.4 to 240.6)	0.44	0.97	167.1
RSMMD	38.0 (27.0 to 64.6)	0.98	1.76 min	45.0	164.2 (116.3 to 278.7)	0.21	1.96	194.0
SDNN	224.6 (159.1 to 381.3)	0.43	1.58	265.4	113.3 (80.3 to 192.4)	0.26	2.28	133.9
PNS index	1.5 (1.1 to 2.5)	0.98	15.27	1.8	5.7 (4.0 to 9.7)	0.25	9.31	6.7
SNS index	0.4 (0.3 to 0.7)	0.97	24.97	0.5	0.7 (0.5 to 1.1)	0.61	9.63	0.8
Mean HR	3.1 (2.2 to 5.3)	0.91	8.72	3.7	7.2 (5.1 to 12.3)	0.59	3.76	8.5

Note. ICC interpretation= Value below 0.5: poor reliability; values between 0.5 and 0.75: moderate reliability; values between 0.75 and 0.9: good reliability; values above 0.9 indicate excellent reliability. CVs of $\leq$10% and $\leq$5% were considered to represent good and excellent reliability, respectively. Variables description: Tau = Constant time, MRT = Mean Response Time, PNS = Parasympathetic nervous system, SNS = sympathetic nervous system, SDNN = the standard deviation of the R-R intervals, and RMSSD = Root mean square successive differences of R-R intervals.

4.1. Limitations

First, the sample was limited to active participants aged 19 to 30 who engaged in university sports. Future research should confirm the concurrent validity of VOT-NIRS data in other populations, such as elite athletes and various sports, as well as older adults or those with chronic diseases, which could significantly affect resting SmO_2 and HRV data. Additionally, this cross-sectional study requires more exhaustive fatigue monitoring, such as using electromyography or tensiomyography markers, to better understand accumulated fatigue over time. To allow VOT-NIRS to identify chronic fatigue and overtraining, it is essential to monitor acute fatigue phases resulting from training over several weeks and associate them with physical performance markers. Another limitation is the variation in VOT-NIRS protocols, ranging from 30 s to 5 min, with 5 min being the most commonly used [3,63]. We recognize the importance of achieving an ischemic period to activate vasodilatory mechanisms in the muscle and observe the metabolic response. However, similar to other studies, we prefer a 3 min protocol, which is highly correlated with the 5 min period (r = 0.90) [63]. The shorter occlusion time reduces discomfort and the burden on participants from suprasystolic pressures (>220 mmHg) in the vastus lateralis muscle [5]. Another limitation is that NIRS can be sensitive to skin pigmentation, and the data can vary depending on adiposity. These technological errors can be minimized with periodic evaluations of the subjects. Finally, although feedback was provided to participants regarding maintaining habits during measurements, factors such as sleep monitoring, hydration status, and psychological stress—each directly influenced by fatigue—should be addressed more rigorously.

4.2. Future Perspectives

To date, the primary challenge in using VOT-NIRS is the variety of protocols, instruments, and variables employed to measure muscle metabolism. As in other studies, we acknowledge that desaturation reflects the rate of muscle oxygen consumption, indicating oxygen supply capacity, while resaturation refers to microvascular function, showing the ability to return to an aerobic state [53,64]. Along with MRT, these measures can indicate oxidative capacity, similar to phosphocreatine (PCr) restoration [65]. Trained individuals are more efficient in switching between oxidative and anaerobic metabolism in response to ischemia and reperfusion (desaturation and resaturation) [64].

Similar to Koutlas et al. [53], we support the hypothesis that resting arterial occlusion is easy to apply and time-efficient, practical for those who cannot tolerate multiple occlusions or perform exercise. For athletes, it is essential to have diagnostic tests that do not require physical exertion and do not interfere with daily training. HRV is known to be sensitive to acute fatigue changes the following day, but the use of SmO_2 as a fatigue marker is still unclear. Longer follow-up protocols should be established to enhance the reliability of the VOT-NIRS test. For example, changes could be observed every hour, and the test should be repeated on different days and under various environmental conditions to establish normative values. Therefore, researchers must continue to refine their studies to reach a consensus that eliminates controversies surrounding the data and improves practical communication. This research proposes using these variables in future fatigue studies.

5. Conclusions

Acute fatigue increases resting muscle oxygen consumption and accelerated muscle oxygen utilization. This corresponds with increased SNS activation and perceived muscle soreness. Therefore, there is an influence of the autonomic nervous system on muscle oxygenation levels at rest, which can be exacerbated by post-exercise fatigue. Given these findings, VOT-NIRS is a useful tool for assessing acute muscle fatigue and has the potential for evaluating training adaptations.

SmO_2 variables show significant variation, even at rest, but in practice, real changes may be greater than 7% of SmO_2. To avoid confusion due to acute fatigue, changes in desaturation and resaturation should exceed 11% of SmO_2, with slopes ranging from 30% to 70% of SmO_2 during the VOT-NIRS protocol. An advantage of the VOT-NIRS technique is that measurements are taken at rest, eliminating physical exertion for athletes and avoiding interference with daily training. However, testing errors and the influence of fatigue must be considered and addressed in this study.

6. Practical Applications

The practical applications of this study include monitoring fatigue in athletes using VOT-NIRS, which allows for the evaluation of SmO_2 at rest without requiring physical exertion. This could be a strategy to prevent overtraining and optimize recovery. Additionally, SmO_2 data has the advantage of providing information about skeletal muscle oxidative capacity, which has yet to be used to measure post-exercise muscle fatigue. However, researchers must consider the reliability and individual response when using VOT-NIRS in performance and rehabilitation contexts, especially when assessing patients with injuries or cardiovascular conditions. Finally, this study highlights the need for a consensus on the protocols and variables used in future research, improving communication in sports science, and its practical application in training and rehabilitation.

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