



# Side differences and reproducibility of the Moxy muscle oximeter during cycling in trained men

Philip Skotzke<sup>1</sup> · Sascha Schwindling<sup>1</sup> · Tim Meyer<sup>1</sup>

Received: 29 December 2023 / Accepted: 17 May 2024 / Published online: 29 May 2024  
© The Author(s) 2024

## Abstract

**Purpose** Portable near-infrared spectroscopy devices allow measurements of muscle oxygen saturation ( $\text{SmO}_2$ ) in real time and non-invasively. To use NIRS for typical applications including intensity control and load monitoring, the day-to-day variability needs to be known to interpret changes confidently. This study investigates the absolute and relative test–retest reliability of the Moxy Monitor and investigates side differences of  $\text{SmO}_2$  at the vastus lateralis muscle of both legs in cyclists.

**Methods** Twelve trained cyclists and triathletes completed 3 incremental step tests with 5 min step duration starting at 1.0 W/kg with an increase of 0.5 W/kg separated by 2–7 days.  $\text{SmO}_2$  was averaged over the last minute of each stage. For all power outputs, the intra-class coefficient (ICC), the standard error of measurement (SEM) and the minimal detectable change (MDC) were calculated. Dominant and non-dominant leg  $\text{SmO}_2$  were compared using a three-factor ANOVA and limits of agreement (LoA).

**Results** ANOVA showed no significant systematic differences between trials and side. For both legs and all intensities, the ICC ranged from 0.79 to 0.92, the SEM from 5 to 9%  $\text{SmO}_2$  and the MDC from 14 to 18%  $\text{SmO}_2$ . The bias and LoA between both legs were  $-2.0\% \pm 19.9\% \text{SmO}_2$ .

**Conclusion** Relative reliability of  $\text{SmO}_2$  was numerically good to excellent according to current standards. However, it depends on the specific analytical goal whether the test–retest reliability is deemed sufficient. Wide LoA indicate side differences in muscle oxygenation during exercise unexplained by leg dominance.

**Keywords** Muscle oxygenation · Near-infrared spectroscopy · Physiology · Wearable

## Abbreviations

ANOVA	Analysis of variance	NDOM	NIRS device placed on the non-dominant VL
ATT	Adipose tissue thickness	NIRS	Near-infrared spectroscopy
CV	Coefficient of variation	Oxy[heme]	Oxygenated hemoglobin and myoglobin
Deoxy[heme]	Deoxygenated hemoglobin and myoglobin	PPO	Peak power output
DOM	NIRS device placed on the dominant VL	SEM	Standard error of measurement
ICC	Intraclass correlation coefficient	$\text{SmO}_2$	Muscle oxygen saturation
MDC	Minimal detectable change	Total[heme]	Total hemoglobin and myoglobin
		VL	Vastus lateralis muscle
		W/kg	Watt per kilogram bodyweight

Communicated by I. Mark Olfert.

✉ Philip Skotzke  
s8phskot@uni-saarland.de  
Sascha Schwindling  
s.schwindling@mx.uni-saarland.de  
Tim Meyer  
tim.meyer@mx.uni-saarland.de

<sup>1</sup> Institute of Sport and Preventive Medicine, University of Saarland, Campus B8.2, 66123 Saarbrücken, Germany

## Introduction

One of the most important trends in endurance training in the past 10 years has been an increase in both training volume and specific training intensity made possible by a more informed and more precise load-recovery management (Sandbakk et al. 2023). With the rapidly growing field of technology in sports (Sports Tech Research Network 2023),

it is predicted that the use of advanced technologies to improve objective training monitoring will continue to be one of the main trends (Sandbakk et al. 2023). Near-infrared spectroscopy (NIRS) measuring muscle oxygenation can be considered one of these technologies (Perrey 2022). Different to traditional physiological markers like heart rate, lactate or oxygen uptake which assess internal load on a systemic level, NIRS parameters give insight into the balance of oxygen delivery and oxygen demand of specific muscles non-invasively and in real-time (Barstow 2019; Perrey and Ferrari 2018). NIRS utilizes changes in the light absorbing characteristics of hemoglobin and myoglobin when oxygen is bound (Barstow 2019). Thus, oxygenated (oxy[heme]), deoxygenated (deoxy[heme]) and total hemoglobin and myoglobin (total[heme]) can be measured. The relative tissue saturation or muscle oxygen saturation ( $\text{SmO}_2$ ) can be calculated from these parameters (Feldmann et al. 2019).

Apart from lab-graded NIRS devices primarily developed to measure brain oxygenation, commercially available and less expensive portable NIRS devices dedicated to measure muscle oxygen allow the use in real-world settings and everyday training (Perrey and Ferrari 2018). One affordable portable NIRS device is the Moxy Monitor (Fortiori Designs LCC, US). The validity of the Moxy and the 0% to 100% scale has been established in active and passive trails using the arterial occlusion method (Feldmann et al. 2019). Although the Moxy has been used in various studies in applied settings (e.g. Olcina et al. 2019; Paquette et al. 2021; Pratt 2018; Yogeve et al. 2023b), its reliability has not yet been adequately studied. In order to meaningfully implement the Moxy in training, reproducibility of  $\text{SmO}_2$ , bilateral side differences and between-device measurement error need to be known. This is vital for the decision if a change between two tests is “real” or due to measurement or biological error (Chrzanowski-Smith et al. 2020). The validation study from Feldmann et al. (2019) and a few others investigated the test–retest reliability during different activities such as rest, sitting, walking and endurance exercise (Contreras-Briceño et al. 2019; Crum et al. 2017; Gandia-Soriano et al. 2022; McManus et al. 2018; Scholkmann and Scherer-Vrana 2020; Yogeve et al. 2023b, 2023a). Two of those studies looked at the test–retest reliability during cycling and came to different conclusions. Yogeve et al. (2023a) reported good-to-excellent relative reliability and absolute agreement between trials of 5–7%  $\text{SmO}_2$  for different workloads between two incremental cycling tests. Crum et al. (2017) found good reliability for low to moderate intensities, but a greater between-trial variability for higher intensities during two incremental cycling tests using the coefficient of variation (CV). The different results of the two studies can be explained by the different statistical measures used to investigate absolute reliability. For the current study, homoscedasticity has been evaluated and the correct measure for reliability chosen (Atkinson and

Nevill 1998). Another shortcoming is that none of these studies reported if  $\text{SmO}_2$  differs between the vastus lateralis (VL) muscle of both limbs. Previous research reported side differences in power output of 5% to 20% during cycling (Carpes et al. 2010) and a greater deoxy[heme] signal amplitude in the dominant leg during counterweighted single-leg cycling (Iannetta et al. 2019). Reinpöld and Rannama (2023) found low agreement between left and right VL desaturation onset kinetics with no clear relation of these asymmetries to leg dominance. It is unclear if side differences can be observed and if  $\text{SmO}_2$  is different between the dominant and non-dominant leg.

This study sets out the goal to investigate the reproducibility of  $\text{SmO}_2$  measured by a portable near-infrared spectroscopy device at different power outputs between three—instead of the previously investigated two—cycling incremental step tests performed under similar conditions. It aims to provide information to answer two research questions: (1) What is the absolute test–retest reliability of  $\text{SmO}_2$  and what difference in  $\text{SmO}_2$  between two measurements can be considered a real change? (2) Can differences in  $\text{SmO}_2$  between the VL of the dominant and non-dominant leg be observed and what is their magnitude?

## Methods

### Participants

For participant recruitment, a digital information letter was shared with local cycling and triathlon communities and further distributed by word of mouth. A sample of 12 male participants took part in the study ( $31.6 \pm 10.9$  years; body mass:  $78.1 \pm 12.9$  kg; height:  $179 \pm 6$  cm; body fat percentage:  $14.4 \pm 4.6\%$ ; adipose tissue thickness (ATT) left VL:  $5.1 \pm 2.1$  mm; ATT right VL:  $4.9 \pm 2.2$  mm; relative peak power output (PPO):  $4.14 \pm 0.6$  W/kg;  $10.0 \pm 2.5$  h of training per week;  $7.1 \pm 5.0$  years of experience). A required sample size of 10 for the test–retest agreement of  $\text{SmO}_2$  was calculated using the G\*Power software (version 3.9.1.7, Kiel, Germany) with a targeted power of  $\beta=0.8$ ,  $\alpha=0.05$  and a correlation between repeated measures of 0.9 based on the test–retest correlations reported by (Crum et al. 2017). Race experience was required for inclusion. Furthermore, participants had to be healthy and non-smokers. Participants were excluded when taking medication affecting metabolic or cardiovascular performance. The dominant leg was determined using the ball kick test. Seven participants were cyclists, and five participants were triathletes. Based on PPO and training hours, the participants can be classified as *recreationally trained to well-trained* according to the classification proposed by De Pauw et al. (2013). Participants received information regarding the study design and the physical tasks

beforehand. Written informed consent was attained before the first test. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the local Ethics Committee (No. 23-19).

## Design and procedures

### Experimental design

All participants performed three incremental step tests separated by 2–7 days. The tests were performed in the lab of the Institute of Sports and Preventive Medicine of Saarland University at the same time of the day on each occasion ( $\pm 1$  h). The participants were instructed to refrain from fatiguing (long or vigorous) exercise 24 h before the tests and to shave their thighs thoroughly to rule out any impact of body hair on the measurements (Barstow 2019).

### Pre-exercise protocol

At the beginning of the first visit height, body weight, body fat percentage, skinfold thickness at the VL on both legs as well as training and competition history in the sport were assessed. The Moxy devices together with the light shields provided by the manufacturer were placed on the VL of both legs approximately halfway between the greater trochanter and the lateral epicondyle of the femur (Crum et al. 2017). The location of the device was marked using a black permanent marker to ensure identical placement during the following trials.

### Exercise protocol

The cycling step test was performed on the participants own bike mounted on an electronically braked cycle ergometer (Cyclus2, RBM elektronik-automation GmbH, Germany). The protocol started at 1.0 W/kg body weight and every 5 min the resistance was increased by 0.5 W/kg. The test was terminated when voluntary exhaustion was reached. The participants were asked to cycle at their preferred cadence and the supervising sport scientist visually controlled that the same cadence was maintained throughout and between the tests to avoid confounding effects of variable cadence on  $\text{SmO}_2$  (Skovereng et al. 2016). Participants had the option to use an electrical fan for air flow. The settings were replicated between trials to rule out any differences in cooling. The exercise protocol with five-minute stages was chosen to allow attainment of a  $\text{SmO}_2$  steady state. The starting intensity and increments in relation to the body weight were chosen to allow for better comparison between participants.

## Measures

### Adipose tissue thickness

A skinfold caliper (British Indicators LTD, England) was used to access body fat percentage using the sum of 10 skinfolds method (Parizkova 1961) and skinfold thickness at the VL muscle. Adipose tissue thickness (ATT) was calculated as follows: Skinfold thickness  $\times 0.5$  (Barstow 2019).

### Muscle oxygenation

Two portable, commercially available continuous-wave NIRS devices (Moxy Monitor, Fortiori Designs LCC, US) were placed on the VL of the dominant (DOM) and the non-dominant leg (NDOM) to measure  $\text{SmO}_2$ . The standard settings for recording (0.5 Hz, smoothing enabled) were used. Data was recorded on a standard bike computer (Edge 530, Firmware Version 9.73, Garmin, US, Kansas) using two Connect IQ data fields (version 2.14) provided by the manufacturer. The Moxy uses one light emitting diode sequentially sending light waves in four different wavelengths (630–850 nm) into the underlying tissue. 2 detectors, spaced 12.5 mm and 25 mm from the emitter, measure the reflected light and a proprietary algorithm to overcome limitations of the modified Beer-Lambert equation is applied (Feldmann et al. 2019). As continuous wave NIRS relies on the assumption that the differential path length factor and the losses due to scattering are constant, only a quantitative measure of muscle oxygenation can be provided (Barstow 2019). The algorithm is intended to isolate oxygenation of muscle tissue from superficial tissue layers and therefore the term  $\text{SmO}_2$  instead of tissue oxygen saturation is used (Feldmann et al. 2019, 2022).

### Data analysis

The .fit files containing the NIRS data were imported into Golden Cheetah (version 3.6, <https://www.goldencheetah.org>). To compare the last minute of each stage, laps were created for the average  $\text{SmO}_2$  value for DOM and NDOM. All data were entered into SPSS (IBM SPSS Statistics Version 29.0.0.0, IBM, US, New York) for further analysis. All figures were created using R Statistical Software (v4.3.2, R Core Team, 2023) using the ggplot2 package (v3.4.4, Wickham, 2016).

### Statistical analysis

First, to assess absolute reliability, a two-way repeated measures ANOVA was performed to estimate the standard error of measurement (SEM) as the square root from the mean square error term (Atkinson and Nevill 1998; Hopkins 2000;

Weir 2005) for left and right VL. Sphericity was assumed when Mauchly's test returned an  $\alpha > 0.05$ . If sphericity was present, the Greenhouse–Geisser correction was used (Field 2017). For relative reliability, the two-way random intraclass correlation coefficient (ICC) for single scores (model 2,1, based on the nomenclature by Shrout and Fleiss Koo and Li 2016; Weir 2005)) was calculated for each workload and for DOM and NDOM, respectively. The minimal detectable change (MDC) for 95% confidence intervals was calculated using the formula:

$$MDC = SEM \times 1.96 \times \sqrt{2}$$

(Weir 2005). A subgroup analysis for ICC and SEM was performed excluding participants with  $ATT > 7$  mm as it has been shown that adipose tissue thickness above 7 mm has an influence on  $SmO_2$  values (McManus et al. 2018). A three-factor ANOVA (Trial\*Side\*Stage) was used to investigate the differences between DOM and NDOM for each power output. Additionally, the bias and 95% limits of agreement between the  $SmO_2$  values of DOM and NDOM were investigated with the modified Bland–Altman method for repeated measures with varying true values (Bland and Altman 2007). A paired-samples t-test was used to investigate the difference between skinfold thickness at the DOM and NDOM VL. The level of significance was set to  $\alpha = 0.05$  for all tests.

## Results

Figure 1 presents exemplary  $SmO_2$  time course data for one participant and all trials. Two participants repeated one test due to 1) a freeze of the Garmin data fields and 2) large dropouts in data transmission. One data set for

the left and two data sets for the right leg were excluded due to implausible muscle oxygenation kinetics like sudden large drops or increases in  $SmO_2$  that were only present in the  $SmO_2$  data of one leg. These tests could not be repeated due to time constraints of the participants. Furthermore, the data of seven individual stages from three participants had to be excluded due to dropouts. These dropouts occurred at workloads at or above 3.0 W/kg. The remaining data of these tests was still used. In total, 9 out of 76 individual data sets, or 12% of all cases, were either excluded or incomplete. 2 Participants had an  $ATT > 7$  mm and were excluded for additional sub-analysis. The average  $SmO_2$  values for each trial are presented in Figs. 2 and 3 for the left and right VL, respectively.

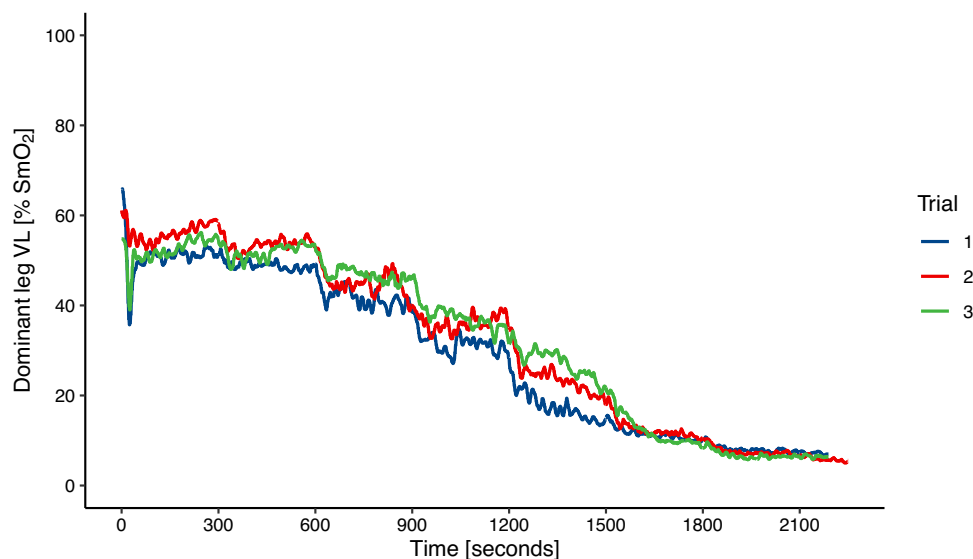
### Absolute reliability

The SEM as well the MDC for each step and left and right VL are presented in Table 1. The SEM ranges from 5–9%  $SmO_2$  with an average SEM of 6%  $SmO_2$ . The MDC ranges from 14 to 21%  $SmO_2$ . Both SEM and MDC were similar for the subgroup analysis.

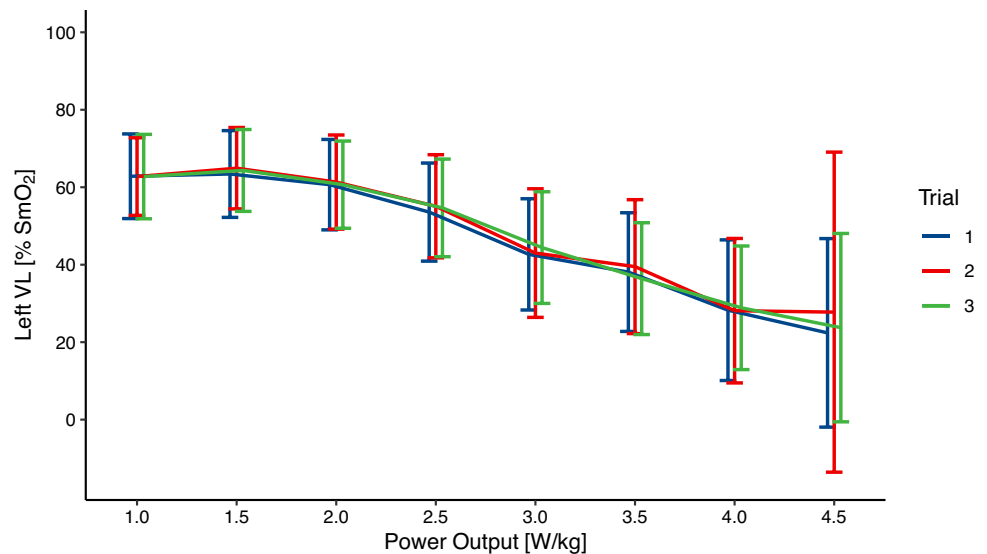
### Relative reliability

The ICCs for the different power outputs and left and right VL are reported in Table 1. The average ICC is 0.89 and individual ICCs range from 0.79 to 0.95 and were lower for lower workloads. The ICC in the sub-group analysis was similar, ranging from 0.78 to 0.95 and being 0.88 on average.

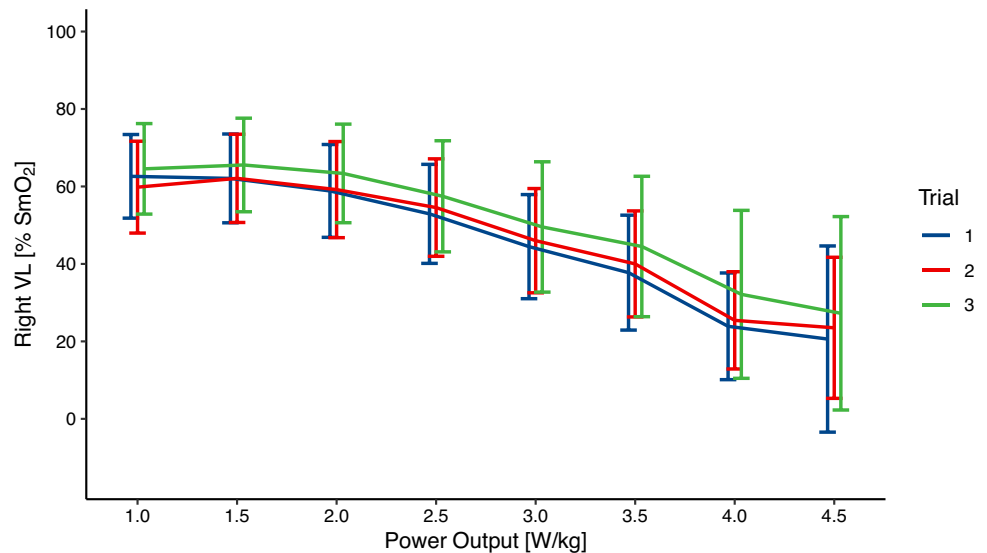
**Fig. 1** Experimental NIRS recordings of one participant.  $SmO_2$  time course data for the dominant leg is presented for all 3 trials



**Fig. 2** SmO<sub>2</sub> values of the left vastus lateralis for each power output and trial. Presented as mean  $\pm$  95% confidence interval



**Fig. 3** SmO<sub>2</sub> values of the right vastus lateralis for each power output and trial. Presented as mean  $\pm$  95% confidence interval



### Comparison of the dominant and non-dominant leg

The difference between the skinfold thickness of the DOM ( $M=10.13$ ,  $SE=1.33$ ) and NDOM ( $M=9.78$ ,  $SE=1.17$ ) VL was 0.36 mm, 95% CI  $[-0.68, 1.40]$  and not significant:  $t(11)=0.754$ ,  $p=0.47$ . The three-factor ANOVA revealed no statistically significant main effects for side and trial as well as no significant interaction effects ( $p>0.05$ ). Figure 4 shows the Bland-Altman plot comparing SmO<sub>2</sub> values between DOM and NDOM. Mean bias was  $-2.0\%$  and 95% confidence limits of agreement adjusted for repeated measures were  $-21.9\%$  and  $+17.9\%$ .

### Discussion

The goal of this study was to investigate the reliability of SmO<sub>2</sub> during cycling at different intensities and compare SmO<sub>2</sub> between the DOM and NDOM leg. It was assumed that the average SmO<sub>2</sub> in the last minute of each stage represents a steady-state behaviour and, thus, measurements can be interpreted as day-to-day variability of SmO<sub>2</sub> during steady state exercise. For practitioners, coaches, and athletes the SEM provides a useful index for the reproducibility. In this study, the SEM ranges from 5 to 9% SmO<sub>2</sub> dependent of the workload and was on average 6% SmO<sub>2</sub>.

**Table 1** Absolute and relative reliability for each power output. 95% Confidence Intervals are provided in square brackets when applicable. Values for sub-analysis are in brackets

Power Output (W/kg)	Side	n	ICC	SEM (% SmO <sub>2</sub> )	MDC (% SmO <sub>2</sub> )
1.0	L	11 (9)	0.79 [0.53, 0.93] (0.86 [0.63, 0.96])	8 (6)	21 (17)
	R	10 (8)	0.87 [0.66, 0.99] (0.78 [0.45, 0.95])	7 (7)	18 (18)
1.5	L	11 (9)	0.84 [0.62, 0.95] (0.87 [0.66, 0.97])	7 (6)	19 (16)
	R	10 (8)	0.88 [0.71, 0.97] (0.79 [0.48, 0.95])	6 (7)	17 (18)
2.0	L	11 (9)	0.90 [0.75, 0.97] (0.90 [0.72, 0.97])	6 (6)	17 (16)
	R	10 (8)	0.94 [0.83, 0.98] (0.90 [0.70, 0.98])	5 (5)	14 (14)
2.5	L	11 (9)	0.92 [0.80, 0.98] (0.93 [0.80, 0.98])	6 (5)	16 (14)
	R	10 (8)	0.94 [0.84, 0.98] (0.91 [0.74, 0.98])	5 (5)	14 (14)
3.0	L	10 (9)	0.90 [0.74, 0.97] (0.90 [0.73, 0.97])	8 (7)	21 (20)
	R	10 (8)	0.94 [0.84, 0.98] (0.92 [0.76, 0.98])	6 (5)	16 (15)
3.5	L	9 (8)	0.89 [0.71, 0.97] (0.89 [0.68, 0.97])	8 (8)	21 (22)
	R	9 (7)	0.94 [0.82, 0.98] (0.91 [0.71, 0.98])	6 (6)	17 (16)
4.0	L	7 (7)	0.85 [0.57, 0.97] (0.85 [0.57, 0.97])	9 (9)	24 (24)
	R	6 (6)	0.89 [0.61, 0.98] (0.89 [0.61, 0.98])	6 (6)	18 (18)
4.5	L	4 (4)	0.92 [0.61, 0.99] (0.92 [0.61, 0.97])	6 (6)	16 (16)
	R	4 (4)	0.83 [0.32, 0.99] (0.83 [0.32, 0.99])	7 (7)	18 (18)
Average			0.89 [0.68, 0.97] (0.88 [0.64, 0.95])	6 (6)	18 (17)

*L* left vastus lateralis, *R* right vastus lateralis, *ICC* intraclass correlation coefficient, *SEM* standard error of measurement, *MDC* minimal detectable change, *SmO<sub>2</sub>* muscle oxygenation

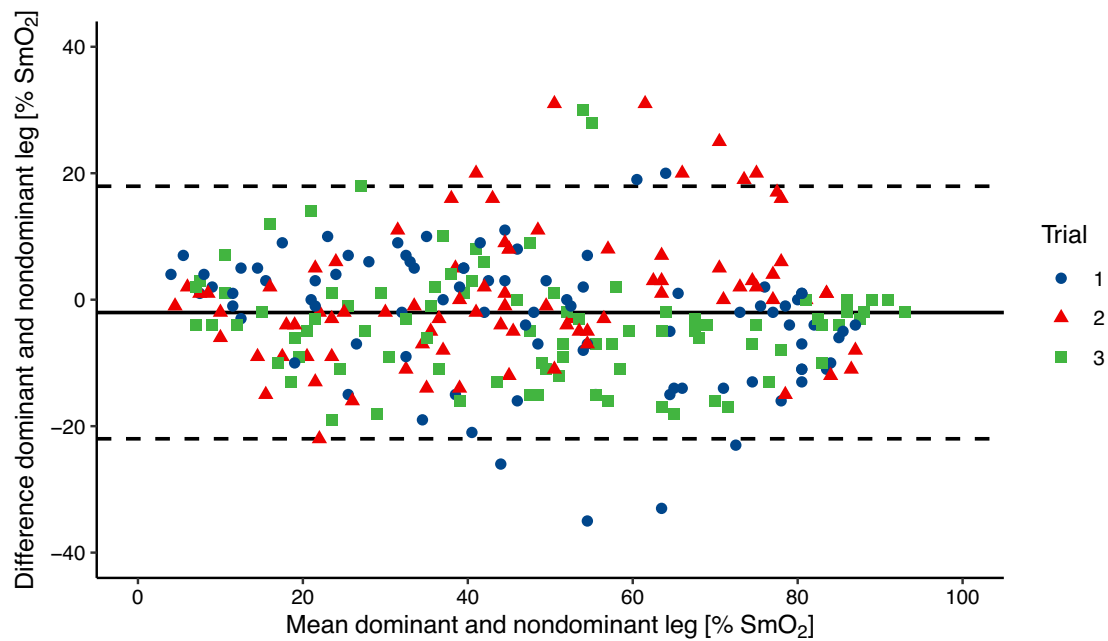
This finding is highly similar to the reported SEM of Yogeve et al. (2023a) of 5–7% SmO<sub>2</sub> for standardized workloads for two similar, but intermittent cycling tests. The finding that the absolute reliability is similar during different intensities is in contrast to the results reported by Crum et al. (2017), who observed an increase in CV with increasing power output. However, The CV should be used when heteroscedasticity is present (Atkinson and Nevill 1998) and is less suitable for homoscedastic data as present here. After testing for heteroscedasticity, the SEM was chosen as more appropriate measure. Yogeve et al. (2023a) also concluded that the SEM is more suited for homoscedastic SmO<sub>2</sub> data.

The ICC reflects the ability to differentiate between individuals (Weir 2005) and using typical cut-offs, the ICCs obtained in this study, ranging from 0.78 to 0.95, indicate good to excellent relative reliability (Koo and Li 2016). The

ICC values obtained in this study are very similar to the values reported by Crum et al. (2017) of 0.77–0.92 as well as Yogeve et al. (2023a) of 0.81–0.90. Contreras-Briceño et al. (2019) state comparable ICCs for an incremental running test (0.95–0.97 for the VL and 0.84–0.93 for the intercostal muscles).

The MDC can be used to decide if an observed difference between two measurements can be considered real (Weir 2005). In this study the MDC ranges from 14 to 21% SmO<sub>2</sub> and was on average 18% SmO<sub>2</sub>, implying that one can say with 95% certainty that the difference between two measurements under similar conditions is real if the SmO<sub>2</sub> value for a specific power output differs by at least 18% SmO<sub>2</sub>. When considering directional changes, e.g. improved muscle oxygenation at the same power output, Hopkins (2000) illustrates that using 95% confidence intervals result in a





**Fig. 4** Bland-Altman plot comparing the average  $\text{SmO}_2$  value of the last minute of the dominant and non-dominant leg VL for all participants and completed stages ( $-2.0\% \pm 19.9\%$  (bias  $\pm$  LoA))

97.5% probability that the improvement is real. He points out that this amount of certainty is impractical in high-performance sports as it circumvents making any decisions for future training modifications. Applying this rationale, using a difference of half the MDC leaves an 84% probability that the improvement is real. In this case, depending on the workload, a meaningful difference would be in the range of 7–11%.

After excluding two participants with ATT values between 7.7 and 8.8 mm at the VL, both ICC and SEM remain mostly unchanged. Due to the small number of participants with ATT values only slightly above the maximum recommended value of 7 mm by McManus et al. (2018), no prediction can be made whether higher ATT has an impact on the reliability of muscle oxygenation at the VL during steady-state cycling. While ATT mostly explained between-subject differences at rest in their study, the impact of ATT on reliability was not investigated.

If NIRS can be used to delineate different power outputs, it could be used to prescribe and control exercise intensities. Between the power outputs of 1.0 to 2.0 W/kg the average  $\text{SmO}_2$  was almost constant (see Figs. 2, 3). At the same time, the SEM is about 7%  $\text{SmO}_2$  and therefore higher than the differences between 0.5 W/kg different power outputs. Between the power outputs of 2.0 W/kg to 4.0 W/kg the average  $\text{SmO}_2$  value drops by about 10% between stages while the SEM is approximately 6–7%. Between the last two stages the difference in  $\text{SmO}_2$  is smaller and around 4%. This is slightly smaller than the SEM of around 6% for these power outputs. This means that the SEM is higher than the difference in  $\text{SmO}_2$  between stages for low and high power

outputs. In this sample, for power outputs in the range of 2.5 to 4.0 W/kg the SEM is smaller than the difference between mean  $\text{SmO}_2$  values, indicating that in this range  $\text{SmO}_2$  can be better used to differentiate between power outputs. However, due to different levels in fitness between participants, it is not possible to draw conclusions about the exercise intensity domain at these workloads. Similarly, Bonilla et al. (2022) did not find a difference in  $\text{SmO}_2$  between neighbouring steps of a graded exercise test, but  $\text{SmO}_2$  was significant different between maximal fat oxidation, the first as well as the second ventilatory threshold.

In addition to the investigation of the reproducibility of  $\text{SmO}_2$ , left and right  $\text{SmO}_2$  were compared to explore side differences. No systematic difference between DOM and NDOM was detected. This is confirmed by the Bland-Altman plot showing a small bias of 2% lower  $\text{SmO}_2$  for the dominant leg. However, the wide limits of agreement ( $\sim \pm 20\% \text{ SmO}_2$ ) show that left and right values can differ substantially. No significant side differences of  $\text{SmO}_2$  are in contrast with reported bilateral differences in power output and a reported roughly 25% higher deoxy[heme] amplitude in the dominant leg during ramp tests (Iannetta et al. 2019). Similar to our findings, Reinpöld and Rannama (2023) found that differences in bilateral desaturation onset kinetics were unrelated to leg dominance. The results indicate that leg dominance does not explain side differences in  $\text{SmO}_2$ . The unexplained differences could be explained by measurement error. As the same device used was on the same leg in this study, further studies are needed to investigate if this is due to a between-device error or biological variability.

One unexpected finding of this investigation was that 12% of the individual data sets were incomplete or had to be excluded completely. Possible reasons for the observed dropouts are movement artifacts or tissue ischaemia as pointed out by Crum et al. (2017) or interference in the wireless data transmission. Using a bike computer placed in close proximity to the rider (~ 1 m) to record the data replicates how the devices typically would be used. Anecdotally, no dropouts during outdoor cycling were observed with the same bike computer. Based on the loss of data in  $\geq 10\%$  of cases it can be recommended to use a second Moxy on the opposite limb as a backup in case of faulty or missing data.

## Limitations

This study is, as any research, not without limitations. First, a small sample of only 12 participants was used (Atkinson and Nevill 1998). The findings need to be replicated with a larger number of subjects to further investigate differences between ATT and muscle oxygenation. Future studies should also include female participants as female athletes typically have higher adipose tissue thickness than men (McManus et al. 2018). Some research exists indicating that Moxy-derived  $\text{SmO}_2$  and its kinetics differ between sexes (Espinoso-Ramírez et al. 2021; Sendra-Pérez et al. 2023), but the effect of sex or higher ATT on the reliability of  $\text{SmO}_2$  remains unclear. Nevertheless, to the authors best knowledge this is the first study investigating the reliability of the Moxy device using 3 trials. The training and exercise regime between trials was not strictly controlled, which might have impacted the reliability negatively. In turn, this could also be considered a strength of the study design, as it might better reflect real-world conditions where not every training session is performed well rested and under identical conditions. One major limitation is that the results of this study can not be transferred to different sports or muscles. Finally, only  $\text{SmO}_2$  was investigated, as it has a higher practical relevance than total[heme], which also seems to be harder to interpret (Barstow 2019). Future studies should try to answer if different Moxy devices can be used interchangeably and if the  $\text{SmO}_2$  values of the opposite limbs are comparable.

## Conclusion

This study demonstrates that Moxy-derived muscle oxygenation values during an incremental cycling test are associated with good-to-excellent relative reliability determined using the ICC and an average SEM of 6%  $\text{SmO}_2$ . These results are in line with previous research investigating the test–retest reliability of the Moxy device. Acceptability of the SEM as a measure of reproducibility can only be assessed with respect to the analytical goal. When the goal is to target a

specific intensity, the Moxy was only able to delineate 0.5 W/kg differences between 2 and 4 W/kg in this sample. Thus, it cannot be recommended to use the absolute  $\text{SmO}_2$  value measured by Moxy for a precise intensity control. In order to detect changes in  $\text{SmO}_2$  between two measurements, a difference of at least 9%  $\text{SmO}_2$  needs to be observed to consider the improvement real with an 84% probability. Wide limits of agreement for side differences were detected in this sample, which could not be explained by leg dominance. Practitioners have to be cautious comparing  $\text{SmO}_2$  values between dominant and non-dominant leg VL. Therefore, it can be recommended to use the same device placed on the same leg and muscle to reduce the impact of between-device and side-specific differences.

**Acknowledgements** Not applicable.

**Author contributions** All authors contributed the conception and design of the experiment. Material preparation, data collection and analysis were performed by Philip Skotzke. The first draft of the manuscript was written by Philip Skotzke and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Funding** Open Access funding enabled and organized by Projekt DEAL.

**Data availability** The datasets generated during, and the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors have no relevant financial or non-financial interests to disclose.

**Ethical approval** This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Saarland University (6 September 2023/No. 23-19).

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

## References

- Atkinson G, Nevill AM (1998) Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Med* 26(4):217–238. <https://doi.org/10.2165/00007256-199826040-00002>
- Barstow TJ (2019) Understanding near infrared spectroscopy and its application to skeletal muscle research. *J Appl Physiol* 126(5):1360–1376. <https://doi.org/10.1152/jappphysiol.00166.2018>



- Bland JM, Altman DG (2007) Agreement between methods of measurement with multiple observations per individual. *J Biopharm Stat* 17(4):571–582. <https://doi.org/10.1080/10543400701329422>
- Bonilla AV, González-Custodio A, Timón R, Cardenosa A, Camacho-Cardenosa M, Olcina G (2022) Training zones through muscle oxygen saturation during a graded exercise test in cyclists and triathletes. *Biol Sport* 40(2):439–448. <https://doi.org/10.5114/biolSport.2023.114288>
- Carpes FP, Mota CB, Faria IE (2010) On the bilateral asymmetry during running and cycling—review considering leg preference. *Phys Ther Sport Off J Assoc Chart Physiother Sports Med* 11(4):136–142. <https://doi.org/10.1016/j.ptsp.2010.06.005>
- Chrzanowski-Smith OJ, Piatrikova E, Betts JA, Williams S, Gonzalez JT (2020) Variability in exercise physiology: Can capturing intra-individual variation help better understand true inter-individual responses? *Eur J Sport Sci* 20(4):452–460. <https://doi.org/10.1080/17461391.2019.1655100>
- Contreras-Briceño F, Espinosa-Ramirez M, Hevia G, Llambias D, Carrasco M, Cerda F, López-Fuenzalida A, García P, Gabrielli L, Viscor G (2019) Reliability of NIRS portable device for measuring intercostal muscles oxygenation during exercise. *J Sports Sci* 37(23):2653–2659. <https://doi.org/10.1080/02640414.2019.1653422>
- Crum EM, O'Connor WJ, Van Loo L, Valckx M, Stannard SR (2017) Validity and reliability of the Moxy oxygen monitor during incremental cycling exercise. *Eur J Sport Sci* 17(8):1037–1043. <https://doi.org/10.1080/17461391.2017.1330899>
- De Pauw K, Roelands B, Cheung SS, De Geus B, Rietjens G, Meeusen R (2013) Guidelines to classify subject groups in sport-science research. *Int J Sports Physiol Perform* 8(2):111–122. <https://doi.org/10.1123/ijssp.8.2.111>
- Espinosa-Ramírez M, Moya-Gallardo E, Araya-Román F, Riquelme-Sánchez S, Rodríguez-García G, Reid WD, Viscor G, Araneda OF, Gabrielli L, Contreras-Briceño F (2021) Sex-Differences in the Oxygenation Levels of Intercostal and Vastus Lateralis Muscles During Incremental Exercise. *Front Physiol* 12. <https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2021.738063>. Accessed 13 Feb 2024
- Feldmann A, Schmitz R, Erlacher D (2019) Near-infrared spectroscopy-derived muscle oxygen saturation on a 0% to 100% scale: reliability and validity of the moxy monitor. *J Biomed Opt* 24(11):115001. <https://doi.org/10.1117/1.JBO.24.11.115001>
- Feldmann A, Ammann L, Gächter F, Zibung M, Erlacher D (2022) Muscle oxygen saturation breakpoints reflect ventilatory thresholds in both cycling and running. *J Hum Kinet* 83(1):87–97. <https://doi.org/10.2478/hukin-2022-0054>
- Field A (2017) *Discovering statistics using IBM SPSS statistics*, 5th edn. SAGE Publications
- Gandia-Soriano A, Salas-Montoro JA, Javaloyes A, Lorente-Casaus C, Zabala M, Priego-Quesada JJ, Mateo March M (2022) Validity and reliability of two near-infrared spectroscopy devices to measure resting hemoglobin in elite cyclists. *Int J Sports Med* 43(10):875–880. <https://doi.org/10.1055/a-1828-8499>
- Hopkins WG (2000) Measures of reliability in sports medicine and science. *Sports Med* 30(1):1–15. <https://doi.org/10.2165/00007256-200030010-00001>
- Iannetta D, Passfield L, Qahtani A, MacInnis MJ, Murias JM (2019) Interlimb differences in parameters of aerobic function and local profiles of deoxygenation during double-leg and counterweighted single-leg cycling. *Am J Physiol Regul Integr Comp Physiol* 317(6):R840–R851. <https://doi.org/10.1152/ajpregu.00164.2019>
- Koo TK, Li MY (2016) A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med* 15(2):155–163. <https://doi.org/10.1016/j.jcm.2016.02.012>
- McManus CJ, Collison J, Cooper CE (2018) Performance comparison of the MOXY and portaMon near-infrared spectroscopy muscle oximeters at rest and during exercise. *J Biomed Opt* 23(1):1. <https://doi.org/10.1117/1.JBO.23.1.015007>
- Olcina G, Perez-Sousa MÁ, Escobar-Alvarez JA, Timón R (2019) Effects of cycling on subsequent running performance, stride length, and muscle oxygen saturation in triathletes. *Sports* 7(5):115. <https://doi.org/10.3390/sports7050115>
- Paquette M, Bieuzen F, Billaut F (2021) The effect of HIIT vs. SIT on muscle oxygenation in trained sprint kayakers. *Eur J Appl Physiol* 121(10):2743–2759. <https://doi.org/10.1007/s00421-021-04743-z>
- Parizkova J (1961) Total body fat and skinfold thickness in children. *Metabolism* 10:794–807
- Perrey S, Ferrari M (2018) Muscle oximetry in sports science: a systematic review. *Sports Med (auckland, n.z.)* 48(3):597–616. <https://doi.org/10.1007/s40279-017-0820-1>
- Perrey S (2022) Muscle oxygenation unlocks the secrets of physiological responses to exercise: time to exploit it in the training monitoring. *Front Sports Act Living*. <https://doi.org/10.3389/fspor.2022.864825>
- Pratt C (2018) Muscle oxygenation patterns during a maximal incremental cycling and 20- km time trials [Master's thesis]. University of Wisoncion - La Crosse
- Reinhold K, Rannama I (2023) Oxygen uptake and bilaterally measured vastus lateralis muscle oxygen desaturation kinetics in well-trained endurance cyclists. *J Funct Morphol Kinesiol*. <https://doi.org/10.3390/jfmk8020064>
- Sandbakk Ø, Pyne DB, McGawley K, Foster C, Talsnes RK, Solli GS, Millet GP, Seiler S, Laursen PB, Haugen T, Tønnessen E, Wilber R, van Erp T, Stellingwerff T, Holmberg H-C, Sandbakk SB (2023) The evolution of world-class endurance training: the scientist's view on current and future trends. *Int J Sports Physiol Perform*. <https://doi.org/10.1123/ijssp.2023-0131>. (**<Emphasis Type="Italic">published online ahead of print 2023</Emphasis>**)
- Scholkmann F, Scherer-Vrana A (2020) Comparison of two NIRS tissue oximeters (mox and nimo) for non-invasive assessment of muscle oxygenation and perfusion. *Adv Exp Med Biol* 1232:253–259. [https://doi.org/10.1007/978-3-030-34461-0\\_32](https://doi.org/10.1007/978-3-030-34461-0_32)
- Sendra-Pérez C, Priego-Quesada JJ, Salvador-Palmer R, Murias JM, Encarnacion-Martinez A (2023) Sex-related differences in profiles of muscle oxygen saturation of different muscles in trained cyclists during graded cycling exercise. *J Appl Physiol* 135(5):1092–1101. <https://doi.org/10.1152/jappphysiol.00420.2023>
- Skovereng K, Ettema G, van Beekvelt MCP (2016) Oxygenation, local muscle oxygen consumption and joint specific power in cycling: The effect of cadence at a constant external work rate. *Eur J Appl Physiol* 116(6):1207–1217. <https://doi.org/10.1007/s00421-016-3379-x>
- Sports Tech Research Network (2023) White Paper: Quality Framework for Sports Technologies—A standardized, evidence-based decision-making framework for evaluating the value, usability, and quality of sports technology. <https://strn.co/special-interest-group>. Accessed 23 July 2023
- Weir JP (2005) Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. *J Strength Cond Res* 19(1):231–240. <https://doi.org/10.1519/15184.1>
- Yogev A, Arnold J, Nelson H, Clarke DC, Guenette JA, Sporer BC, Koehle MS (2023a) Comparing the reliability of muscle oxygen saturation with common performance and physiological markers across cycling exercise intensity. *Front Sports Act Living*. <https://doi.org/10.3389/fspor.2023.1143393>
- Yogev A, Arnold J, Nelson H, Clarke DC, Guenette JA, Sporer BC, Koehle MS (2023b) The effect of severe intensity bouts on muscle oxygen saturation responses in trained cyclists. *Front Sports Act Living*. <https://doi.org/10.3389/fspor.2023.1086227>