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ORIGINAL ARTICLE

Reproducibility and sex differences in muscle oxygenation during brachial artery occlusion in healthy participants

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Abstract

Significance: Near-infrared spectroscopy (NIRS) measurement is a widely used technique to measure muscle oxygenation. A knowledge of the reproducibility of NIRS measurements is essential for the correct interpretation of data.

Aim: Our aim was to test the reproducibility and sex differences of NIRS measurements during brachial artery occlusion in healthy participants.

Approach: An NIRS device was used to measure muscle oxygenation and microvascular function during a 5 min brachial occlusion. Muscle oxygen consumption (mVO_2) and tissue saturation index (TSI%) were used. The occlusion test was performed three times on separate days for males (n = 13, 28 ± 8 years) and females (n = 13, 29 ± 7 years).

Results: During the occlusion phase, the reproducibility of mVO_2 was excellent (intraclass correlation; ICC = 0.90). During the reperfusion phase, the maximal change in TSI% revealed the best reproducibility (ICC = 0.77). There were no sex differences in reproducibility. Male participants had higher muscle oxygenation during occlusion (mVO_2, 0.054 ± 0.010 vs. 0.038 ± 0.012 mLO_2/min/100 g, p = 0.001, male and female, respectively). There were no sex differences during the reperfusion phase.

Conclusion: The reproducibility of NIRS to measure muscle oxygenation and microvascular function during circulation occlusion and reperfusion is good to excellent. Muscle oxygen capacity measured during occlusion is higher in males compared to females, and there are no sex differences in microvascular function during the reperfusion phase.

KEYWORDS
microcirculation, muscle oxygenation, reproducibility, sex difference, skeletal muscle

1 | INTRODUCTION

Microcirculation is a network of blood vessels <150 μm in diameter, comprising arterioles, capillaries and venules. This network is responsible for the primary function of the vascular tree and the regulation of tissue perfusion for optimal exchange of gases and removal of metabolic waste products (Smits et al., 2016). Near-infrared spectroscopy (NIRS) is a widely used technique allowing the noninvasive measurement of skeletal muscle microcirculation at rest and during exercise. The NIRS device measures oxyhemoglobin,

deoxyhemoglobin, total haemoglobin concentration changes and tissue saturation index (TSI), all of which can be used as measures of skeletal muscle tissue oxygenation and microcirculation function (Boone, Vrabas, et al., 2016; Boone, Vandekerckhove, et al., 2016). Tissue oxygen concentration changes reveal muscle oxygen metabolism: the ability of skeletal muscle oxygen utilization and oxygen delivery to skeletal muscle (Jones et al., 2016). Mitochondria play an important role in skeletal muscle cell energy metabolism by producing adenosine triphosphate from oxygen-dependent oxidative phosphorylation (Willingham & McCully, 2017). Thus, by measuring TSI, NIRS also provides information about muscle oxidative metabolism and capacity for oxidative phosphorylation (Ryan et al., 2014).

Recent studies have proved that impaired muscle oxygenation (measured by NIRS) is linked to several cardiovascular diseases such as hypertension (Dipla, Triantafyllou, Koletsos, et al., 2017), heart failure (Southern et al., 2015) and diabetes mellitus (Dipla, Triantafyllou, Grigoriadou, et al., 2017; Stephens et al., 2023). Patients with hypertension (Dipla, Triantafyllou, Koletsos, et al., 2017) or heart failure (Southern et al., 2015) have low skeletal muscle oxidative capacity and reduced microvascular reactivity compared to healthy individuals (Dipla, Triantafyllou, Koletsos, et al., 2017; Southern et al., 2015). In addition, women with gestational diabetes (Dipla, Triantafyllou, Grigoriadou, et al., 2017) and patients with diagnosed type 1 or 2 diabetes (Stephens et al., 2023) have alterations in muscle oxygen delivery, utilization and microvascular responsiveness during exercise compared to healthy subjects (Dipla, Triantafyllou, Grigoriadou, et al., 2017; Stephens et al., 2023). These studies have demonstrated that small alterations in microvascular structure, vasomotor tone and endothelial dysfunction suggest that microvascular changes may lead to significant macrovascular changes, potentially resulting in the development of hypertension and other cardiovascular diseases. NIRS technology has also been used to show impaired muscle oxidative metabolism in other diseases, such as Parkinson's disease (Sharma et al., 2021) and cystic fibrosis (Erickson et al., 2015). In addition, NIRS has been utilized to study athletes' oxygen levels and exercise responses (Perrey & Ferrari, 2018). These studies have proved that NIRS measurements can help us to learn about microvascular function and skeletal muscle oxygenation, and therefore, it is important to establish that this technique is highly reliable.

To our knowledge, only a few studies have tested the reproducibility of NIRS during brachial artery occlusion (Lacroix et al., 2012; Rogers et al., 2023). One study tested reproducibility by measuring oxyhemoglobin and deoxyhemoglobin during brachial artery occlusion, but it did not assess reproducibility for TSI, and the participants involved were exclusively male (Lacroix et al., 2012). Another study tested the effect of age on microvascular reactivity and reproducibility, but only in females (Rogers et al., 2023). Other studies have tested the reproducibility of NIRS during handgrip exercises (Celie et al., 2012) and compared NIRS to flow-mediated dilation measurements (FMD), which is a different method to measure vascular reactivity (Mclay, Fontana, et al., 2016). One study has also tested reproducibility by measuring different NIRS parameters at the tibialis anterior muscle during different occlusion durations (Iannetta et al., 2019). These studies have shown that NIRS is a good and reliable technique for testing microvascular function and skeletal muscle oxygenation. Two recent studies have used NIRS techniques to test the difference in microvascular reactivity and muscle oxygenation between male and female participants (Dellinger et al., 2023; Rasica et al., 2022). Rasica et al. found sex differences during both occlusion and reperfusion phases, whereas Dellinger et al. reported sex differences only in the reperfusion phase. (Contrary to our protocol, both studies measured NIRS variables at the vastus lateralis muscle.) Our aim is to test the reproducibility of TSI measured by NIRS during brachial artery occlusion in healthy males and females and to compare muscle oxygenation and reperfusion between males and females.

2 | METHODS

2.1 | Participants

Twenty-six young healthy voluntary female (n = 13) and male (n = 13) participants with a wide range of aerobic capacity (all performing leisure-time aerobic exercise more than two times/week) were recruited in the study (medical students and their friends). Candidates were included if they were nonsmoking, 20–40 years and did not have any cardiovascular risk factors or diseases. Exclusion criteria were as follows: BMI > 30, blood pressure >130/85 mmHg (home monitoring for 7 days) or use of any medication. The participants were told to refrain from any vigorous exercise and alcohol consumption 2 days before testing. The study was conducted according to the Declaration of Helsinki and approved by the ethical committee of the Northern Ostrobothnia Hospital District in Oulu, Finland. All participants provided written informed consent. The characteristics of the participants are presented in Table 1.

2.2 | Protocol

The participants visited our laboratory (Research Unit of Biomedicine and Internal Medicine, University of Oulu) four times within a 2-month period, with at least 2 days between each visit. During the first visit, the participants performed anthropometrics, body fat % (InBody 720 Biospace), and 5 min brachial arterial occlusion tests (with NIRS measurements) as a familiarization setup. In addition, blood haemoglobin from the fingertip (Hemocue Hb 201+ Hemocue AB) and maximal oxygen uptake (Vyntus CPX; Vyaire Medical) by incremental bicycle ergometer (Monark 839E) until exhaustion were performed. After the initial measurements, blood pressure monitors (Omron M6 Comfort) were given to the participants to measure their blood pressure at home, three times in the morning and evening for 7 days. During the second, third and fourth visits, a brachial artery occlusion test was performed always at the same hour of the day for each subject (between 8 AM to 5 PM).

TABLE 1 Baseline characteristics of study subjects.

	Male $n = 13$	Range Minimum–maximum	Female $n = 13$	Range Minimum–maximum	p Value
Age, years	28 (8)	21–43	29 (7)	22–47	0.694
Weight, kg	81 (10)	63–95	62 (5)	54–74	<0.001
Height, cm	180 (7)	162–188	168 (3)	163–172	<0.001
BMI, kg/m^2	25 (3)	21–30	22 (2)	20–25	0.003
Fat, %	15 (6)	7–25	21 (5)	15–30	0.005
Muscle mass, %	49 (3)	43–54	44 (3)	38–49	<0.001
Hb, g/L	147 (9)	130–158	126 (8)	109–138	<0.001
Systolic BP, mmHg	118 (5)	103–124	108 (11)	88–125	0.011
Diastolic BP, mmHg	72 (9)	58–85	67 (5)	60–76	0.090
Mean BP, mmHg	87 (7)	76–99	80 (6)	71–89	0.017
Exercise capacity					
Load max, W	277 (39)	220–360	202 (27)	175–265	<0.001
Vo_{2max}, mL/kg/min	47 (7)	36–60	42 (5)	35–50	0.065
Heart rate max, bpm	193 (8)	176–203	192 (10)	166–210	0.669
RER	1.11 (0.04)	1.04–1.19	1.13 (0.03)	1.07–1.17	0.225

2.3 | NIRS measurements

Skeletal muscle oxygenation and microvascular function were measured with a NIRS device (Oxymon MK III; Artinis Medical System) by placing NIRS optodes in the superficial muscle of the left forearm's flexor digitorum. Data was recorded in the same controlled quiet room and the entering light in the room was minimized. The measurement point was selected at a location where the melanin concentration was low, and the point was drawn on transparent paper for each person to ensure the same measurement point for each test and to prevent NIRS signal loss due to skin melanin concentration (Barstow, 2019). The optodes were attached to the skin of the participants with adhesive stickers so that the angle and position of the optodes were kept constant. By measuring relative changes in light absorption at different wavelengths, oxygenated haemoglobin (O_2Hb) and deoxygenated haemoglobin (HHb) were measured; TSI [$O_2Hb/(HHb+O_2Hb)$] was automatically calculated by an ARTENIS device algorithm as an absolute parameter for muscle oxygenation (Rosenberry et al., 2018). Before every measurement, the NIRS device (2-channel) was calibrated and the participant was seated, left arm supported at resting position. TSI calibration was performed before every experiment according to manufacturer specifications. A TSI calibration basket was used for TSI calibrations. The device (Oxymon) was turned on at least 20 min before the calibrations. Then the receiver and transmitter fibres were connected to the TSI optode holder. The optode holder was placed at the top of the calibration basket, the device settings were set, and the calibration was automatically executed by the software. An automated pneumatic cuff inflator (Hokanson Model E20) was positioned above the left elbow on the arm to induce rapid blood flow occlusion. After a 1 min period of baseline measurements, the cuff pressure was rapidly inflated to 250 mmHg for 5 min (occlusion period) followed by a rapid deflation (reperfusion period) that lasted 3 min. During the occlusion period, total haemoglobin ($O_2Hb + HHb$) was monitored to verify stable blood volume. NIRS signals were sampled at 10 Hz and transformed to 1 Hz, from which all data was analyzed.

2.4 | NIRS parameters

Baseline TSI values (TSI base) were defined as an average of the last 30 s during the 60 s baseline. The minimum and maximum TSI values (TSI min and TSI max) were analyzed and utilized to compute TSI changes during and after occlusion (ΔTSI base-min, ΔTSI max-min, ΔTSI max-base). Based on the literature (Lacroix et al., 2012; Rogers et al., 2023), the following parameters were considered to reveal muscle oxidative capacity and O_2 utilization during occlusion: muscle oxygen consumption (mVO_2, $mLO_2/min/100\,g$) (calculated during the occlusion period), slope during the 5 min occlusion (Occlusion slope, %/s) and ΔTSI base-min. Slope during the first 15 s of reperfusion (Reperfusion slope, %/s) together with ΔTSI max-min and ΔTSI max-base were utilized as measures of microvascular reactivity, which is defined as the capacity of blood vessels to accommodate increased blood flow (Dipla, Triantafyllou, Koletsos, et al., 2017). The reactive hyperaemic area under the curve was calculated in two different ways (AUC-1, AUC-1+2). AUC-1 is the total area under the reperfusion curve above the TSI base value after the 2 min cuff

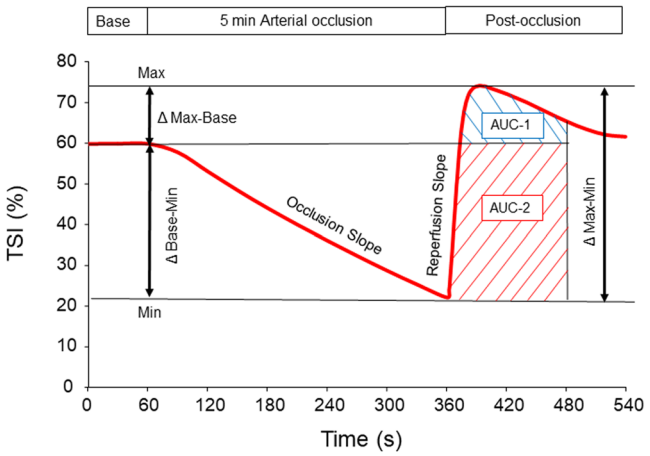

FIGURE 1 An example of tissue saturation index (TSI) dynamics at rest, during 5 min occlusion and 3 min reperfusion phases for one subject.

release (Dellinger et al., 2023; Rasica et al., 2022). AUC-1+2 was calculated from the area under the curve after a 2 min cuff release above the TSI min value (Rosenberry et al., 2018). The first visit was treated as familiarization, and the results of the last three visits were analyzed. The definition for each parameter is shown in Figure 1.

2.5 | Statistical analyses

The data was analyzed using SPSS software (IBM SPSS Statistics 24; IBM Corp.). The data is reported as mean ± standard deviation, and a *p*-value of <0.05 was considered significant. The dependent variables were checked for normality (Gaussian distribution) by visual inspection and the Shapiro–Wilk test. The NIRS values were individual averages over three measurements for every subject, and differences between males and females were assessed by independent *t*-tests. One-way-ANOVA was used to compare each NIRS parameter between the three tests. Reproducibility was analyzed using the intraclass correlation coefficient (ICC) (Koo & Li, 2016). The reproducibility was defined as poor if the ICC was ≤0.20, fair if between 0.21 and 0.40, satisfactory if between 0.40 and 0.61, good if between 0.61 and 0.80 and excellent if the ICC was ≥0.81 (Altman, 1990). The reproducibility of every parameter was also estimated using the coefficient of variation (CV) (SD/average of parameter × 100), and the standard error of measurement (SEM) was calculated as recommended by Hopkins (2000) (SEM % = SEM/average of every measurement × 100).

3 | RESULTS

3.1 | Baseline characteristics

The baseline characteristics of the participants are shown in Table 1. Significant differences based on sex were observed for weight ($p < 0.001$), height ($p < 0.001$), BMI ($p = 0.003$), body fat % ($p = 0.005$), blood haemoglobin ($p < 0.001$), systolic ($p = 0.011$) and mean blood pressure ($p < 0.017$) and maximal exercise load ($p < 0.001$).

3.2 | Reproducibility

The measured NIRS parameters during the three tests are presented in Table 2. There were no significant differences between the tests. Table 3 describes the reproducibility of each NIRS parameter. The reproducibility of most parameters ranged from good to excellent (ICC: 0.64–0.90). TSI base, ΔTSI max-base and AUC-1 were the least repeatable, but still satisfactory (ICC: 0.44–0.56). Comparing the absolute repeatability between males and females using CV, for example, occlusion slope ($p = 0.67$) and reperfusion slope ($p = 0.91$) showed that there was no significant difference in the measurements of males and females.

3.3 | Sex differences

The dynamics of TSI during occlusion and reperfusion phases (separately for males and females) are shown in Figure 2 and the absolute values of NIRS parameters are in Table 4. There were significant sex differences during the occlusion phase in all NIRS parameters—mVO_2 ($p = 0.001$), ΔTSI base-min ($p = 0.012$) and occlusion slope ($p = 0.021$)—with all parameters revealing differences in muscle oxidative capacity between male and female participants (Figure 2a). There were no sex differences during the reperfusion phase in any parameters revealing muscle microvascular function (Figure 2b).

4 | DISCUSSION

The present study shows that the reproducibility of muscle oxidative capacity measured by NIRS techniques during brachial artery occlusion with several parameters varies from good to excellent. The reproducibility of muscle microvascular function measured during the reperfusion phase after occlusion varies from satisfactory to good. Our study also shows that there was no difference in the reproducibility between male and female participants. While muscle oxidative capacity measured during brachial artery occlusion was higher in male compared to female subjects, there were no differences between sexes in the reperfusion phase with respect to markers of muscle microvascular function.

4.1 | Reproducibility

During the occlusion period, the repeatability of each NIRS parameter was at least good, and muscle oxygen consumption (mVO_2) was the best parameter to measure muscle oxidative capacity, having excellent reproducibility. The same was observed in a previous

TABLE 2 NIRS parameters were measured during occlusion and post-occlusive reactive hyperaemia.

	Test-1	Test-2	Test-3	*p* Value	Physiology
mVO_2 (mLO_2/min/100 g)	0.047 (0.014)	0.046 (0.015)	0.046 (0.015)	0.91	Muscle oxidative capacity
Occlusion slope (%/s)	−0.130 (0.028)	−0.128 (0.031)	−0.128 (0.031)	0.95	
ΔTSI base-min (%)	39.1 (7.7)	38.9 (9.4)	38.6 (9.2)	0.98	
Reperfusion slope (%/s)	3.06 (0.97)	3.16 (0.92)	3.13 (0.86)	0.92	Muscle microvascular function
ΔTSI max-min (%)	54.4 (11.0)	53.9 (12.1)	53.6 (11.6)	0.97	
ΔTSI max-base (%)	15.2 (5.4)	15.0 (4.1)	15.0 (4.6)	0.98	
AUC-1 (a.u.)	1170 (423)	1161 (416)	1206 (356)	0.91	
AUC-1+2 (a.u.)	5632 (920)	5510 (1128)	5520 (1084)	0.90	
TSI base (%)	59.3 (6.0)	58.9 (4.9)	60.2 (5.5)	0.68	Baseline and response
TSI min (%)	20.2 (9.7)	20.0 (11.8)	21.6 (12.8)	0.86	
TSI max (%)	74.5 (3.8)	73.9 (5.0)	75.2 (3.6)	0.53	

Abbreviations: NIRS, near-infrared spectroscopy; TSI, tissue saturation index.

TABLE 3 Reproducibility evaluation of NIRS parameters during occlusion and post-occlusive reactive hyperaemia based on ICC as excellent, good and satisfactory.

	ICC	CV (%)	SEM (%)	Evaluation	Physiology
mVO_2 (mLO_2/min/100 g)	0.90	13.5	9.9	Excellent	Muscle oxidative capacity
Occlusion slope (%/s)	0.78	13.6	10.8	Good	
ΔTSI base-min (%)	0.78	13.6	10.5	Good	
Reperfusion slope (%/s)	0.71	19.8	15.7	Good	Muscle microvascular function
ΔTSI max-min (%)	0.77	12.6	10.1	Good	
ΔTSI max-base (%)	0.51	24.0	21.8	Satisfactory	
AUC-1 (a.u.)	0.44	25.3	25.0	Satisfactory	
AUC-1+2 (a.u.)	0.73	11.4	9.7	Good	
TSI base (%)	0.56	6.9	6.1	Satisfactory	Baseline and response
TSI min (%)	0.79	42.8	25.4	Good	
TSI max (%)	0.64	3.8	3.3	Good	

Abbreviations: CV, coefficient of variation; ICC, intraclass correlation; NIRS, near-infrared spectroscopy; SEM, standard error of measurement; TSI, tissue saturation index.

study, where mVO_2 was found to be the most repeatable parameter during the occlusion period (Lacroix et al., 2012). In addition, mVO_2 is commonly known as the most used and 'golden standard' parameter for measuring muscle oxygen consumption, metabolic rate and thus muscle oxidative metabolism, especially under circulatory occlusion (Abozguia et al., 2008). On the other hand, the reproducibility of the other parameters, such as occlusion slope and ΔTSI base-min, have also been shown to be good (Rogers et al., 2023). It is also important to note that a longer occlusion period leads to better repeatability, and thus, the assessment of muscle oxygenation may be more reliable under such conditions (Iannetta et al., 2019).

ΔTSI max-min, reperfusion slope and AUC-1+2 were the best parameters for evaluating muscle microvascular function during the reperfusion phase. The reproducibility of all these parameters was good, with the ΔTSI max-min ICC value being the best. Again, a recent study has shown similar results during the reperfusion period (Rogers et al., 2023). Reperfusion Slope has also been a very repeatable parameter when measured at the tibialis anterior muscle. In addition, the reproducibility of NIRS technology has been shown to be higher than FMD techniques when studying vascular function (Mclay, Fontana, et al., 2016). Reperfusion slope also correlated with FMD, which has been used in the past to evaluate vascular endothelial function (Mclay, Nederveen, et al., 2016). This proves that NIRS technology is a very reproducible method compared to previous methods that evaluate muscle microvascular function during the reperfusion phase.

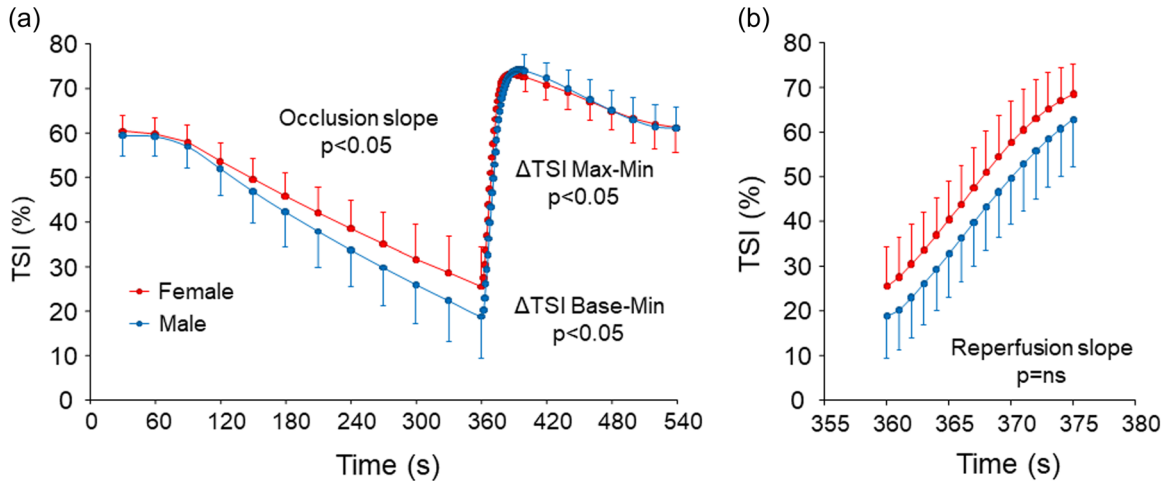

FIGURE 2 The dynamics of tissue saturation index (TSI) during occlusion (a) and reperfusion (b) separately for males (blue line) and females (red line).

TABLE 4 Difference in NIRS parameters during occlusion and post-occlusive reactive hyperaemia between males and females.

	Male	Female	*p* Value	Physiology
mVO_2 (mLO_2/min/100 g)	0.054 (0.010)	0.038 (0.012)	0.001	Muscle oxidative capacity
Occlusion slope (%/s)	−0.140 (0.020)	−0.118 (0.025)	0.021	
ΔTSI base-min (%)	42.3 (5.4)	35.4 (7.4)	0.012	
Reperfusion slope (%/s)	3.12 (0.73)	3.12 (0.76)	0.984	Muscle microvascular function
ΔTSI max-min (%)	58.2 (7.6)	49.7 (9.6)	0.020	
ΔTSI max-base (%)	15.9 (3.5)	14.3 (3.1)	0.238	
AUC-1 (a.u.)	1218 (293)	1139 (252)	0.469	
AUC-1+2 (a.u.)	5934 (705)	5120 (888)	0.016	
TSI base (%)	59.3 (4.6)	59.7 (3.5)	0.774	Baseline and response
TSI min (%)	16.9 (9.2)	24.3 (8.7)	0.048	
TSI max (%)	75.1 (3.2)	74.0 (3.2)	0.380	

Abbreviations: NIRS, near-infrared spectroscopy; TSI, tissue saturation index.

It should be mentioned that only a few other studies have examined the reproducibility of NIRS parameters during the occlusion and the reperfusion periods. These studies have tested only men (Iannetta et al., 2019; Lacroix et al., 2012; Mclay, Nederveen, et al., 2016) or women (Rogers et al., 2023), and to our knowledge, ours is the very first study to prove that there is no difference in reproducibility between men and women. In addition, our results strongly suggest that the NIRS technique is highly repeatable in measuring different parameters and evaluating skeletal muscle oxidative capacity and microvascular function.

4.2 | Sex differences

In the present study, our primary finding was that there was a significant difference between males and females during the occlusion period, but not during the reperfusion period. Male participants had a greater mVO_2 and ΔTSI base-min (%). In addition, occlusion slope for males was also steeper compared to females. Previous studies have reported similar results during the occlusion period (Dellinger et al., 2023; Rasica et al., 2022). There was a significant difference in occlusion slope between sexes (Rasica et al., 2022), and males had a greater ΔTSI base-min (%) compared to females (Dellinger et al., 2023). These findings show that males had better muscle oxidative capacity during the occlusion period, which might be due to their having a greater muscle mass compared to females in general. Males, thus, have more functioning mitochondria to consume oxygen during the occlusion period.

The second finding of this study was that there was no significant difference between the sexes during the reperfusion period. Existing studies have implied that there might be a sex

difference in the reperfusion period too (Dellinger et al., 2023; Rasica et al., 2022). It is noteworthy that these studies tested microvascular function at the vastus lateralis muscle. Fellahi et al. (2014) have shown that results change significantly depending on the anatomical location of the measurement. In their study, males had a greater reperfusion slope at the proximal leg, but the same measurement at the distal foot for females had a greater reperfusion slope compared to males (Fellahi et al., 2014). In addition, studies that have tested muscle macrovascular function using FMD have obtained results similar to those of our study. These studies showed that there was not a significant difference between sexes in microvascular reactivity in arm muscle (Jensen-Urstad, 2001; Nishiyama et al., 2008). There are many factors that can explain the different results in microvascular reactivity during the reperfusion period. Both anatomical blood vessel size (Schroeder et al., 2000) and relative muscle mass disparity between the arms and legs (Nishiyama et al., 2008) may cause differences during the reperfusion period, and hence differences in microvascular function. Due to these factors, more research is needed to explain possible sex differences in the reperfusion phase and to better understand skeletal muscle microvascular function in different parts of the human body.

5 | CONCLUSIONS

Several conclusions can be drawn from our findings. Firstly, the reproducibility of muscle oxidative capacity during brachial artery occlusion is excellently measured by modern NIRS techniques. Secondly, the reproducibility of muscle microvascular function measured during the reperfusion phase after occlusion is good to satisfactory. Thirdly, there were no sex differences in the reproducibility of NIRS parameters during or after occlusion. Finally, males had a higher muscle oxidative capacity measured during occlusion than females, but there were no sex differences in the muscle microvascular function during the reperfusion phase.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this article are not publicly available due to privacy and ethical concerns. They can be requested from the author at mikko.tulppo@oulu.fi.

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