mNIRS muscle oxygenation conference

Book of Abstracts

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Muscle oxygenation and its use to study mitochondrial capacity

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Abstract:
Near Infrared Spectroscopy (NIRS) has been used to evaluate skeletal muscle (mNIRS) for at least 87 years. The aim of this presentation is to address three current issues in the use of NIRS to evaluate skeletal muscle: quantification of oxygen levels, assessing reactive hyperemia, and assessing muscle mitochondria. NIRS signals include hemoglobin and myoglobin which have different oxygen saturation curves, the proportion of which influences NIRS based resting oxygen levels. In addition, NIRS involves shining light into a tissue that includes skin and adipose tissue as well as skeletal muscle. Quantifying NIRS signals using a physiological calibration (ischemic hypoxia and subsequent reperfusion) will be discussed. An important aspect of muscle function is related to oxygen consumption, and the use of recovery (offset) kinetics to measure muscle mitochondrial capacity will be discussed. Discussion of mitochondrial capacity measurements will include theoretical considerations, various measurement protocols, validation experiments, and examples of applications. As the application of mNIRS to the study skeletal muscle becomes more widespread, and as technological advances in mNIRS technology improve, it will become increasingly important to understand how to use and interpret mNIRS signals. mNIRS has the potential to become an essential part of studies that involve understanding and evaluating skeletal muscle at rest and during exercise, and in athletic as well in patient populations.

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NIRS developments for mNIRS

Professor Maria Franceschini

(3)

Muscle near-infrared Spectroscopy (mNIRS) in healthcare and observational cohort studies

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Abstract:
Skeletal muscle is important for physical function, metabolic homeostasis and thermogenesis. Muscle function declines with ageing and many diseases negatively impact muscle physiology. Near-infrared Spectroscopy (NIRS) provides an opportunity to non-invasively assess several key features of skeletal muscle physiology, including oxidative respiratory performance (capacity), microvascular vasodilatory capacity and oxygenation changes in response to exercise. Muscle NIRS (mNIRS) has been used in healthcare research to help understand disease mechanisms, assess response to interventions and to understand muscle changes with ageing. However, despite potential utility in a clinical setting, such as identification of pathophysiology, tracking disease progression and treatment responses, mNIRS techniques are not routinely applied in clinical healthcare. Barriers to clinical uptake may include lack of understanding around the technology, interpretation of analysis outcomes and lack of clinical thresholds.

The first aim of this presentation is to describe examples of mNIRS application in healthcare research and highlight where these techniques could be useful in clinic, summarising barriers to clinical uptake and potential areas where effort could be made to overcome these. The second aim is to describe examples of mNIRS application in observational cohort studies and highlight the strengths and limitations of mNIRS in this area of research.

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Monitoring in sport has changed: time to consider muscle oxygenation

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Abstract: Internal training load refers to the stress applied on the body during sports practice. Monitoring it is essential as it enables us to assess whether the training is appropriate or excessive for the athlete. The near-infrared spectroscopy (NIRS) technique offers an intriguing possibility for the continuous, real-time, and non-intrusive measurement of human physiologic indicators. Even if the current availability and popularity of wearable NIRS sensors present a promising approach to assessing physical fitness, formal longitudinal and cohort studies are required to ascertain their suitability in the context of sports monitoring.
This presentation will highlight the benefits of utilising the NIRS technique in measuring the muscle physiological status during exercise and training. It will be proposed how the current NIRS devices provide a range of data on the internal load of athletes, which can be used by coaches and practitioners to monitor, evaluate and prescribe training (and competition) characteristics. A number of case studies will be presented, demonstrating the integration of muscle oxygenation metrics alongside the most common methods (i.e., Heart rate and Rating of Perceived Exertion) for the quantification of internal training loads.

Keywords: training, NIRS, muscle, monitoring, internal load.

References:

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 4:864825. doi: 10.3389/fspor.2022.864825.

Perrey S, Quaresima V, Ferrari M. Muscle Oximetry in Sports Science: An Updated Systematic Review. Sports Med. 2024 Apr;54(4):975-996. doi: 10.1007/s40279-023-01987-x.

Muscle Deoxygenation Profiles to Assess Exercise Intensity Domains

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Abstract: Changes in NIRS-derived signals (i.e. deoxygenated hemoglobin/myoglobin (Δdeoxy[heme] and tissue oxygen saturation (StO_2)) have been proposed as valid estimates of muscle oxygen consumption (${}_{m}\dot{V}O_2$). To evaluate the actual metabolic state during exercise, the response-profiles of Δdeoxy[heme] and StO_2 were assessed during cycling in the moderate- heavy- and severe-intensity domain. While StO_2 changed from moderate- to the heavy domain with no further change to the severe domain, there was a systematic change of Δdeoxy[heme] across intensity domains (moderate < heavy < severe).

Methods: Sixteen well-trained cyclists visited the laboratory on three occasions to complete: 1) a graded ramp test to determine maximal measures of power output (P_{max}) and oxygen consumption ($\dot{V}O_{2max}$), and the first ventilatory threshold (VT1); 2+3) 6-min step transitions from baseline to moderate- (90%VT1), heavy- (40% between VT1 and P_{max}) and severe-intensity cycling (70% between VT1 and P_{max}). During all tests, NIRS signals of the right-leg vastus lateralis were measured with a portable continuous-wave NIRS device (PortaMon, Artinis). The step transitions (i.e. 2+3) were averaged to obtain one single transition for each intensity domain. RM-ANOVAs were used to analyse: 1) the maximal change of Δdeoxy[heme] and StO_2 from the last 30-s of baseline cycling for each intensity domain; and 2) the occurrence of a metabolic steady-state within each intensity domain, with the NIRS signals of the step transitions averaged into 1-min bins.

Results: The maximal change of Δdeoxy[heme] was significantly affected by exercise intensity (17.5 ± 7.9 µm, 30.6 ± 10.9 µm and 36.2 ± 13.2 µm for moderate- heavy- and severe intensity, respectively; $F_{1.14;\,17.2} = 72.3$; $p < 0.001$). StO_2 significantly changed from moderate- (8.4 ± 3.5 %) to heavy-intensity (13.4 ± 4.3 %; $p < 0.001$) with no further significant change to severe-intensity (14.4 ± 4.5 %; $p = 0.474$). The Δdeoxy[heme] and StO_2 significantly changed over time within each intensity domain.

Conclusion: The systematic and significant increase of Δdeoxy[heme] across intensity domains, reflect differences in ${}_{m}\dot{V}O_2$ indicative of differences in energy demand. In addition, the response profiles of Δdeoxy[heme] (Figure 1) demonstrated the ability to discriminate ${}_{m}\dot{V}O_2$ across distinctively different exercise intensity domains, extensively reported for pulmonary oxygen consumption or phosphocreatine. After initial increases (2 min) Δdeoxy[heme] decreased, remained stable and increased for the moderate-, heavy- and severe domain, respectively. Whilst oxygen delivery exceeds utilisation in the moderate domain, this was inversed for the severe domain and a balance was observed for the heavy domain. StO_2 however, remained at the same level in the heavy- and severe domain.

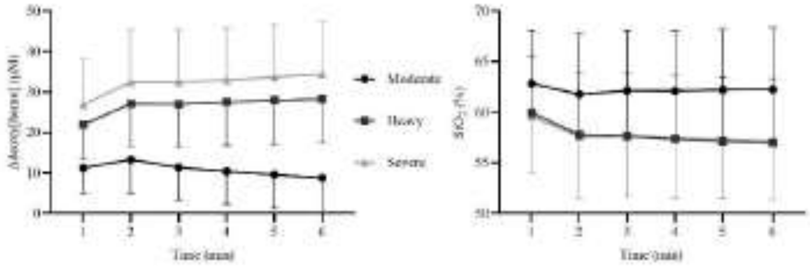

Figure 1: Time course of Δdeoxy[heme] and StO_2 during moderate- heavy- and severe-intensity cycling

Low-cost, Compact, Multiwavelength, and Multidistance Speckle Contrast Optical Spectroscopy System for Non-Invasive Muscle Hemodynamics.

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Abstract: A non-invasive system based on Speckle Contrast Optical Spectroscopy (SCOS) to monitor the hemodynamics of the brachioradialis muscle in real-time [1]. The MMSCOS System is compact (13x22x30 cm), cost-effective, fast (9.8 Hz), and uses multiwavelength (685 nm, 785 nm, 830 nm) and multidistance (12, 17, 20, 25 mm) measurements to assess tissue oxygen saturation (StO2) and blood flow index (BFI). An automated tourniquet for vascular occlusion tests (VOT) and a pulse oximeter for SpO2 monitoring are also included.

Methods: Five healthy volunteers (mean age ~28) participated in a study to evaluate the system performance. Each volunteer underwent baseline measurements, followed by a VOT with the tourniquet inflated for 3 minutes at 50 mmHg above arterial pressure. Ischemia and subsequent reperfusion were monitored for 8 minutes.

Results: The MMSCOS system demonstrates a high signal-to-noise ratio (SNR) when acquiring fast data to resolve pulsatile dynamics (~1.2 Hz). It consistently achieves high SNR across all wavelengths and distances, such as 25.3 dB at 12 mm and 19.3 dB at 25 mm for 685 nm, 21.5 dB at 12 mm and 24.8 dB at 25 mm for 785 nm, and 19.1 dB at 25 mm for 830 nm. The vascular occlusion test results further highlight the system's ability to accurately monitor changes in blood flow index and tissue oxygen saturation in real-time, demonstrating its potential for reliable, non-invasive muscle hemodynamic monitoring.

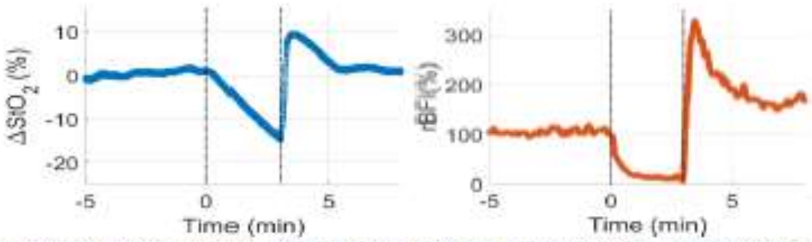

Figure 1. Changes in tissue oxygen saturation (StO2, blue) and relative blood flow index (rBFI, orange) during a vascular occlusion test (VOT).

Conclusion: This study demonstrates the efficacy of the MMSCOS system for non-invasive, real-time monitoring of muscle hemodynamics. Future work will focus on comprehensive comparisons with the established TRS-DCS system to further validate the MMSCOS system performance and reliability.

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[1] C. P. Valdes, Biomed. Opt. Express, BOE, vol. 5, no. 8, pp. 2769–2784, (2014).

Cardiopulmonary fitness and changes in tissue saturation index during exercise in people living with long COVID compared with controls

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Introduction: The pathogenesis of the persistent fatigue and severe exercise intolerance which can follow an infection with the SARS-CoV-2 virus ('Long COVID') is not fully understood.

Methods: Cases (n=31; 56[13]y; 6(19%)men) and controls (n=111; 57[13]y; 26(23%)men) were recruited from two UK population-based cohort studies. We assessed exercise performance using maximal cardiopulmonary exercise testing and skeletal muscle tissue saturation index (TSI) during exercise using near-infrared spectroscopy. Greater drops in TSI during exercise are thought to represent failure of oxygen supply to keep up with demand. Key outcome measures for each physiological system were compared between groups using potential outcome means(95% confidence intervals) adjusted for potential confounders.

Results: When compared to controls and after adjustment for age, sex and BMI, cases reporting fatigue had lower exertional $\dot{V}O_2$peak (21.1(19.3,23.0) ml/kg/min versus 23.3(22.0,24.6) ml/kg/min, p=0.033), oxygen uptake efficiency slope (1823.2(1603.9, 2042.6) ml/min versus 2064.3(1945.5, 2183.2) ml/min, p=0.045), $\dot{V}O_2$ at anaerobic threshold (14.9(13.7, 16.1) ml/kg/min versus 17.1(16.2, 18.1) ml/kg/min, p=0.002) and peak ventilation (49.4(44.8, 54.0) L/min versus 54.6(51.5, 57.6) L/min, p=0.051). Cases and controls had similar TSI values at baseline (67.2(66.0, 68.5) versus 66.1(64.6, 67.7), p=0.292) and end exercise (62.7(60.5, 64.8) versus 61.7(59.5, 63.8), p=0.496). The change in TSI during exercise was more than double in long COVID cases compared to controls (-7.4(-17.4, 2.6) versus, -2.4(-7.4, 2.6) p=0.382), although confidence around the estimates remained wide.

Discussion: Long COVID is associated with reduced measures of exercise performance and a failure of oxygen supply to keep up with demand may contribute to persistent fatigue and exercise intolerance experienced by long COVID sufferers.

Acknowledgements: We are extremely grateful to all the people who took part in this study and to the past and present members of the research team at the Bloomsbury Centre for Clinical Phenotyping at UCL who helped to collect the data.

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An automatized hybrid diffuse optical platform for addressing microvascular health in the skeletal muscle

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ABSTRACT: We present the validation of a fully automated hybrid diffuse optical (hDOS) device that combines time domain near-infrared and diffuse correlation spectroscopies with a pulse oximeter. This device measures synchronized tissue oxygen saturation (StO_2), blood flow index (BFI), peripheral oxygen saturation, and baseline muscle metabolic rate of oxygen consumption ($MMRO_2$). It features an automated inflatable cuff for vascular occlusion tests (VOT), providing additional muscle metabolism indices like deoxygenation rate (DeO_2) and endothelial function indices such as reoxygenation rate (ReO_2) and area under the curve for StO_2 (AUC StO_2) and BFI (AUC BFI). Real-time data quality analysis and display ensures autonomous operation by the end-users even in complex scenarios such as critical care, physical therapy and more.

METHODS: The device has been validated on multiple fronts: *Obj. 1)* assessing accuracy and precision *in vivo* during small alterations of light level and conditions of StO_2 and BFI across different inflation/deflation intervals; *Obj. 2)* testing its usability in the clinic in multiple scenarios. We have also compared hDOS against a commercial medical grade oximeter (INVOS 5100C) in healthy subjects and we addressed its ability to grasp impairments in the microvasculature of patients admitted to the intensive care (ICU) with respect to the healthy population.

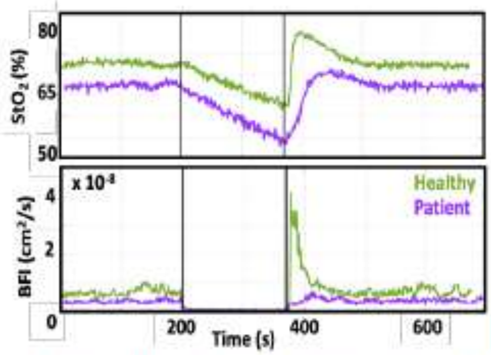

Figure 1 BFI and StO_2 in a healthy (green) and critically ill patient (purple line)

RESULTS: For *Obj.1*, in healthy subjects (N=7), we found that variability depends on mean values across different inflation/deflation intervals. StO_2 and BFI had smaller variability at higher countrates (ρ=-0.5, $p<0.001$ and ρ=-0.41, p=0.001, respectively), as expected. StO_2 precision was better than 4% across the 50%-70% range. BFI variability was higher during full arterial occlusion, consistent with near-zero flow conditions. In pursuit of *Obj.2*, in 10 healthy young subjects we found significantly higher baseline StO_2 (p=0.02) and elevated DeO_2 and ReO_2 ($p<0.001$). The hDOS also displayed two-fold lower intra-subject variability with respect INVOS (3% against 6%) in the baseline. Furthermore, ICU patients (N=96) showed significantly lower baseline values of StO_2, BFI, $MMRO_2$, ReO_2, AUC StO_2 and BFI ($p<0.01$) with respect to controls (N=39) when measured with hDOS (see Figure 1).

CONCLUSION: The hDOS has been validated and continuously improved thanks to more than 300 measurement sessions, contributing to the standardization and the robustness of the platform. Furthermore, hDOS identifies microvascular reactivity impairment during VOT in ICU patients and provides crucial tissue perfusion information, addressing a knowledge gap.

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The last steps of Oxygen cascade: oxygen diffusion and utilization at skeletal muscle level estimated by NIRS

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Aerobic capacity is the strongest predictor of morbidity, mortality and quality of life. Although oxygen delivery to peripheral tissue is a main determinant of maximal oxygen consumption, muscle mitochondria are the final target of the anatomic and physiologic variables supporting oxygen transport during exercise. As such, muscle mitochondrial volume-density and oxidative capacity are strong correlates of endurance exercise performance. However, according to the Fick's law of diffusion, the final steps of the O2 cascade determining muscle O2 uptake depend not only on mitochondria function but also on the product of the microvascular to intramyocyte transmembrane PO2 gradient and the muscle O2 diffusing capacity. This presentation will describe advances in our understanding of the O2 cascade and O2 utilization limitations affecting aerobic capacity in health and chronic disease. An overview of the O2 cascade, emphasizing discoveries from animal models that have set the foundation for human studies, will set the scene. Next, an innovative, non-invasive, near infrared spectroscopy (NIRS) methodology to investigate human skeletal muscle oxidative capacity will be described. The third section of the talk will describe an innovative evolution of the NIRS technique to measure skeletal muscle O2 diffusing capacity and intramuscular O2 flux limitations. This presentation will have a wide target audience, including PhD students, post-doctoral fellows and PI researchers interested in understanding limitations in the O2 delivery and utilization cascade, and innovative techniques to measure how these limitations affect aerobic capacity. The data presented will also be of interest to clinicians, therapists and physiologists working in clinical practice who are interested in understanding how new methods can be introduced in practice and how these relate to understanding exercise intolerance in their patients.

Effect of Leg Pain Intermittent Claudication Symptom Severity in Calf Muscle Oxygen Saturation in Peripheral Artery Disease

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Introduction: Intermittent claudication (IC) characterized by exercise-induced pain due to peripheral arterial disease (PAD), is commonly used as a criterium of improvements related to physical exercise and health benefits in PAD (1). The IC can be unilateral or bilateral, and usually the most symptomatic leg is considered in the evaluation walking tests. This study aims to compare calf oxygen saturation between the most symptomatic leg, and the less symptomatic or no disease leg, at pain-free walking distance (PFWD) and maximal walking distance (MWD) during an incremental protocol, to better understand the exercise-mediated tissue ischemia and the IC related pain. **Methods**: Twenty-two symptomatic men with PAD in the stage IIb of Fontaine classification (claudication at a distance<200m; ankle-brachial index (ABI)<0.9a.u.) were included in this sample (age=65.14±1.3yrs; height=167.1±1.3cm; weight=74.7±2.4kg; most symptomatic leg ABI=0.64±0.1a.u.; less symptomatic leg ABI=0.94±0.2a.u.). During the Gardner-Skinner protocol PFWD, MWD, muscle oxygen saturation (SmO_2) through near-infrared spectroscopy (MOXY, Fortiori Design LLC, Minnesota, USA) and heart rate (H10, Polar Electro, Kempele, Finland) were registered. The average SmO_2 at the first stage (SmO_2avg_1st), at PFWD stage (SmO_2avg_PFWD), at MWD stage (SmO_2avg_MWD), the amplitude of deoxygenation (SmO_2deoxy), and the minimum achieved SmO_2 value (SmO_2min) of the two legs were calculated. **Results**: ANOVA reveal a progressive increase in heart rate, ranging from 82.3±2.8 to 104.8±3.9 bpm (rest and last stage, respectively) with differences between all the stages ($p<0.006$). The t-test revealed no differences between legs in baseline neither for SmO_2 ($p=0.740$) nor SmO_2avg_1st ($p=0.070$). At PFWD and MWD the calf SmO_2 was different between legs ($p<0.001$), with lower SmO_2avg_PFWD values in the most symptomatic leg compared to less symptomatic one (14.4±9.0% and 24.2±10.2%, respectively), and also lower SmO_2avg_MWD values (13.5±9.0% and 22.7±11.1%, respectively). The SmO_2deoxy showed a higher amplitude in the most symptomatic leg compared to the less symptomatic (32.9±11.2 vs 24.5±10.1%, $p=0.004$), and also differences in SmO_2min (12.6±9.0% vs 20.2±9.8%), $t(20)=-4.17$, $p<0.001$, revealing different oxygen kinetics responses between legs with different levels of symptom severity. **Discussion**: These findings suggest that impaired microcirculation related to oxygen delivery and utilisation is higher in the most symptomatic leg and with lower ABI, likely due to underlying dysfunction. Further investigation into the relationship between leg pain, ABI and muscle oxygen saturation is necessary to inform appropriate intervention strategies.

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Analysis methodology for reoxygenation kinetics during an incremental multi-stage cycling protocol

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Introduction: Recovery of muscle oxygen saturation (SmO_2) measured with near-infrared spectroscopy (NIRS) after exercise is often analysed with a monoexponential model. However, real-world kinetics can demonstrate a time delay or 'acceleration phase' before the start of systematic reoxygenation, particularly after higher intensity exercise. We hypothesised that a sigmoidal function would represent reoxygenation kinetics better than a monoexponential function.

Methods: Data were analysed from two separate experiments using an incremental multi-stage cycling protocol with 5-minute work stages separated by 1-min rests, to maximum task tolerance, using different wearable NIRS devices. Twenty-one trained cyclists in Study I performed two trials using the Moxy5 monitor (Fortiori Design LLC, Hutchinson, MN, USA). Thirty trained cyclists in Study II performed one trial using Train.Red+ (Train.Red, Elst, the Netherlands). Reoxygenation kinetics were quantified as: (A) half-recovery time (HRT), (B) mean response time (MRT) of a monoexponential function, and (C) inflection point (Xmid) of a sigmoidal (generalised logistic) function. Goodness-of-fit was compared with R^2 and RMSE at 50, 75, and 100% workloads using a linear mixed-effects model and post-hoc contrasts. Test-retest reliability was evaluated for Study I as intraclass correlation coefficient ($ICC_{2,1}$) and standard error of the measurement (SEM).

Results: Monoexponential and sigmoidal models reached satisfactory fits ($R^2>0.85$) for >90% of available data. There was a significant difference in RMSE in favour of the sigmoidal model only at 100% workload in Moxy ($p<0.05$). No differences were found in R^2. ICC was moderate to good at 50 and 75% workload, and poor at 100% workload. SEM was 1-3 sec for MRT, Xmid, and HRT in Study I with Moxy. There were differences between study datasets in MRT, Xmid, and HRT at 100% workload ($p<0.05$) which may be related to variation between participants or NIRS devices.

Conclusion: In contrast to our hypothesis, both monoexponential and sigmoidal functions suitably modelled free-flow postexercise reoxygenation kinetics after cycling exercise above 50% intensity in datasets with two different wearable NIRS devices. Within-participant variability and differences between study datasets were largest after maximal intensity.

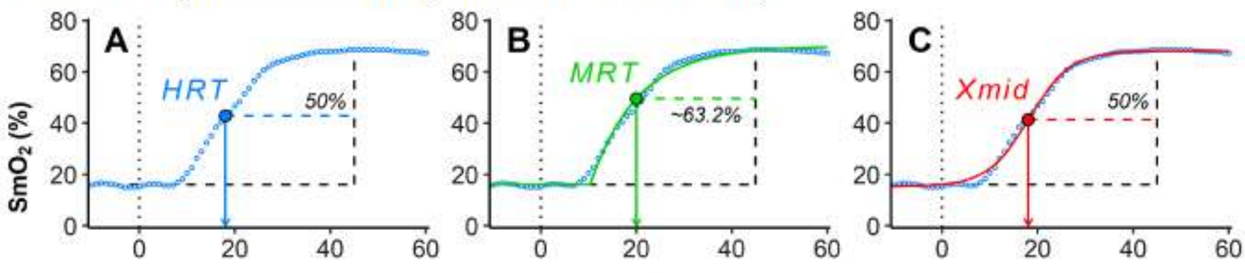

Figure 1 Representative reoxygenation kinetics after a work interval (time = 0) quantified as: (A) Half-recovery time. (B) Monoexponential MRT. (C) Sigmoidal Xmid.

(12)

The impact of skinfold thickness and exercise intensity on the reliability of NIRS in the vastus lateralis.

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Introduction: The aims of this study were (1) to assess the test-retest reliability of the primary near-infrared spectroscopy (NIRS) variables (i.e., StO_2, T[Hb], [HbO_2] and [HHb]) during cycling and (2) to investigate potential influences of exercise intensity and adipose tissue thickness (ATT) on this reliability.

Methods: 21 men and 20 women completed twelve constant work rate tests (6 min) at six different exercise intensities with each intensity performed twice. NIRS (Oxiplex TS, ISS, Champaign, IL, United States) variables were measured at the vastus lateralis. The coefficient of variance (CV%), the intraclass correlation coefficient (ICC), mean bias and limits of agreement (LoA) were determined for reliability purposes.

Results: The reliability of baseline values was acceptable to very good (CV% range: 5.83 – 21.96%). The reliability of end-values (CV% range: 0.02 – 25.02%, ICC range: 0.0 – 0.935) and amplitudes (ICC range: 0.0 – 0.887) were more variable. In general, the mean biases of end-values and amplitudes showed wide limits of agreement. A homogeneous influence of exercise intensity on reliability could not be established but reliability measures appeared to be lower in people with a lower skinfold thickness. Moreover, the NIRS signals decreased with increasing ATT but stabilized upon reaching a cut-off of 8 mm ATT. In addition, ATT did have a significant influence on [HHb] amplitude. In participants with ATT $<$ 8 mm, higher amplitudes were observed with increasing intensity whereas in participants with ATT $>$ 8 mm, there were no differences between the intensities.

Discussion: The study reveals variable results with regards to reliability and there was no consistent influence of exercise intensity on reliability. Participants with a lower skinfold thickness showed stronger reliability. Moreover, NIRS signals decrease when ATT exceeds 8 mm. Careful consideration is necessary when interpreting NIRS signals in such cases.

The effect of position of a near-infrared spectroscopy (NIRS) device on measurements of oxidative capacity and post-occlusive reactive hyperaemia (PORH)

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Background: Skeletal muscle function is an often overlooked yet valuable area providing insight into cardiovascular, metabolic and neuromuscular health. Near-infrared spectroscopy (NIRS) can be combined with vascular occlusions to assess skeletal muscle oxygen consumption, oxidative capacity, and microvascular function. However, the effect of device positioning within the same muscle has received limited investigation.

Aims: This study aims to compare metabolic and vascular measures using NIRS from 2 positions on the lateral gastrocnemius: 1) the largest cross-sectional area of the muscle (L1) and 2) 2cm below this point (L2).

Methods: Participants were recruited from a university student population. Arterial cuff occlusion techniques were coupled with a continuous wave NIRS (CW-NIRS) device, measuring from the left lateral gastrocnemius. Skeletal muscle measures for oxygen consumption (resting muscle $\dot{V}O_2$), oxidative capacity (a time constant for post-exercise muscle $\dot{V}O_2$ recovery), and microvascular function (post-occlusive reactive hyperaemia [PORH]) were determined. NIRS signals were processed using custom-written scripts (MATLAB). Summary statistics are mean±SD or n(%). Correlation was assessed via Spearman's correlation coefficients (r_s), and Bland-Altman analysis was conducted to estimate the mean difference and limits of agreement (LoA) between measures in the two positions.

Results: Young, healthy participants (n=14; 20.9±0.95 years; 7(50%) men) underwent NIRS measurements. Resting muscle $\dot{V}O_2$ between positions L1 and L2 displayed a moderate to strong positive correlation (r_s=0.61, $p<0.05$). There was a small mean difference between the measures but wide LoA (-0.025 [LoA: -0.17,0.12] ΔHbdiff μM/s). The time constants between positions L1 and L2 displayed a strong positive correlation (r_s=0.71, p=0.007). There was a small mean difference and wide LoA (-1.75 [LoA: -23.64, 20.10] s). The time to 95% PORH between positions L1 and L2 demonstrated a very weak positive correlation (r_s=0.16, p=0.59). Although there was a small mean difference, LoA were wide (-0.721 [LoA: -13.415, 11.972] s).

Conclusion: Oxidative capacity and oxygen consumption measurements using CW-NIRS are less strongly influenced by device positioning than PORH measurements. There was no systematic bias detected through re-positioning the NIRS device, however, the wide confidence limits of agreement around all of our estimates suggests high variance in these measurements. It will be important to verify these findings in a larger sample size.

(14)

Two-month Combined Exercise Improves Glycemic Control, Physical Function and Muscle Oxidative Capacity in Older adults with Type 2 Diabetes

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Introduction: Exercise training, particularly resistance and endurance exercises, has the potential to enhance muscle oxygen extraction, microvascular blood flow, and oxygen delivery to muscles in older adults. Combined exercise can improve resting substrate oxidation, insulin sensitivity, and motor function in older adults with Type 2 diabetes (T2D). However, the impact of these factors on muscle oxygen levels (muscle oxygenation, SmO_2) remains incompletely understood. This study aims to investigate the effects of combined exercise on glycemic control, physical function, and mitochondrial and microvascular function in those with T2D.

Methods: This experimental study analyzed data from 20 sedentary older adults with T2D who underwent a 2-month exercise intervention. The exercise regimen consisted of personalized progressive exercises conducted six days a week. Glycated hemoglobin (HbA1c) was assessed using the A1CNow+ device. Muscle function was evaluated using the Bilateral Heel Raise Test (BHR), while SmO_2 was measured during and after the BHR test via Moxy device. Desaturation rate (negative) during the BHR test is calculated via a liner regression, indicating mitochondrial function. Microvascular function was assessed using the resaturation rate (positive) after the BHR test.

Results: After 2 months of combined exercise, those with T2D showed a significant improvement in HbA1c, which reduced from 7.15% (SD=1.0) to 6.91% (SD=1.0) (p=.0095). The exercise regimen also improved the score of the BHR test although the improvement was not significant. Notably, the desaturation rate during the BHR test increased from -0.60 (SD=0.3) to -0.91 (SD=0.6) (p=.0048), indicating better mitochondrial function. Similarly, the resaturation rate after the BHR test during reperfusion accelerated from 0.25 (SD=0.1) to 0.33 (SD=0.2) (p=.0181), indicating better microvascular function.

Discussion: Our study revealed a significant reduction in HbA1c levels among participants with T2D after the 2-month intervention, with mean levels falling below the 7% target threshold associated with preventing micro- and macrovascular complications related to diabetes. This finding aligns with prior research. Furthermore, improvements in physical function observed in our study are consistent with previous literature, suggesting that a combination of aerobic and resistance training can enhance glycemic control and physical function in older adults with T2D. Notably, our study is the first to demonstrate improvements in muscle oxidative capacity following exercise intervention in this population, filling a gap in the existing literature.

Conclusion: The two-month combined exercise program yielded notable benefits in glycemic control and physical function, partially attributed to enhanced mitochondrial and microvascular function. This personalized exercise regimen is promising for future application in community and home settings.

The Effects of 4-Weeks of Ischaemic Preconditioning at the Ankle on the Oxygenation and Reperfusion of the Foot

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Introduction: Ischaemic preconditioning (IPC) involves repeated cycles of alternating occlusion and reperfusion, more recently adopted prior to exercise. Currently, most studies use IPC during single sessions or during 7-day repeat interventions, with fewer studies applying chronic interventions over longer timeframes. The aim of this study was to assess the use of a 4-week IPC intervention performed at the ankle using near-infrared spectroscopy (NIRS) to assess the health of the foot. Furthermore, the localised response at the foot was compared to the whole-body oxygen consumption response during an isolated incremental step test involving isotonic resistance exercise on the isokinetic dynamometer.

Method: With institutional ethical approval, 28 participants volunteered to participate (19 males, 9 females; age: 25.3 ± 10.2 years; height: 173.6 ± 9.4 cm; body mass: 72.7 ± 13.6 kg). Participants visited for a pre- and post-testing session involving an incremental step test performing 30 repetitions of isotonic plantarflexion and dorsiflexion at an increasing resistance on the isokinetic dynamometer. Participants were randomly assigned to one of four groups: IPC, SHAM, IPC followed by exercise or exercise only. The training sessions involved 2 sessions per week for 4-weeks with three occlusion cycles of 5-minutes at an individualised arterial occlusion pressure (Average: 225 mmHg) for the IPC groups and 20 mmHg for the sham groups. The exercise groups performed 3 sets of 8 repetitions at 80% of peak torque during the incremental test. The health of the foot was assessed during every session using NIRS (moorVMS-NIRS, Moor Instruments Ltd, Axminster, UK) to assess the haemoglobin concentration and reoxygenation rate in the abductor hallucis muscle.

Results: There was no significant difference in the maximum torque achieved during pre-testing and post-testing ($p > 0.05$). Further, there were no significant differences between pre- and post-testing or between groups for VO_{2peak} ($F(3,24) = 0.477$, $p = 0.701$), SO_{2max} ($F(3,24) = 0.100$, $p = 0.959$), O_2Hb_{max} ($F(3,24) = 0.045$, $p = 0.987$) or HHb_{max} ($F(3,24) = 0.413$, $p = 0.745$). The average reoxygenation rate for SO_2 was 0.43 ± 0.25% for pre-testing and 0.56 ± 0.47% for post-testing. While there were no significant differences in the reoxygenation rate overall for the NIRS variables, there were some differences when assessing the reoxygenation and haemoglobin responses by workload, but these were not consistent across workloads or variables.

Discussion: The aim of this intervention was to assess whether 4-weeks of IPC resulted in any improvement in NIRS-derived variables as an indicator of the health of the foot in a healthy population. Despite the results not evidencing an improvement, this study included healthy participants, so it is feasible that no improvements were evident as the participants presented with a healthy vascular status. This study is one of the first known to apply a repeated IPC intervention with an ankle occlusion, which offers an isolated foot intervention with minimal pain. This could offer an IPC protocol for people who are unable to exercise due to lower limb injuries or health conditions, such as in people living with diabetes and diabetic foot.

Changes in microvascular responsiveness after step reduction and retraining in young subjects

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Introduction: Inactivity is an important and independent risk factor for all-cause mortality, and there is increasing evidence about the importance of minimizing sedentary behavior to reduce the risk of cardiovascular diseases[1]. Endothelial dysfunction, classically investigated as changes in macro- and microvascular responsiveness, plays an important role in the pathogeneses of cardiovascular diseases, being directly linked with cardiovascular morbidity and mortality[2]. It has been previously shown that bed-rest, a classical model utilized to study the physiological effects of severe inactivity, has a negative impact on the endothelial function[3,4]. The effects of step reduction, a milder form of inactivity, on the microvascular responsiveness are currently unclear. Moreover, it is not known whether an impaired microvascular responsiveness resulting from the steps reduction would differently recover after either endurance or resistance training. In the present study, we investigate the effects of 14 days of step reduction and subsequent retraining program on the microvascular responsiveness by near-infrared spectroscopy(NIRS).

Methods: Fifteen (8 males and 7 females) young subjects (age: 22±2,7ys, weight: 69±10kg, height: 172±6cm) were assessed before (PRE), after 14 days of step reduction (SR), and after a 3-week retraining program consisting of either nine sessions of moderate-intensity (85%W_{GET}) cycling (END;n=7) or nine sessions of lower-limb resistance exercises (70%1RM) (RES;n=8). Daily steps were monitored by a waist-worn accelerometer. Microvascular responsiveness was estimated by monitoring changes of tissue saturation index (TSI) at vastus lateralis muscle by NIRS, during a vascular occlusion test consisting of a prolonged ischemia followed by a reperfusion phase[6]. The rate of muscle deoxygenation during the first minute of occlusion (Slope1) was used as proxy of resting muscle oxygen uptake. Time of ischemia (t_{isch}) and the lowest TSI value reached during occlusion (TSI_{min}) were calculated to estimate the ischemic vasodilatory stimulus. The rate of muscle reperfusion during the first 10s ($Slope2_{10}$) was assessed and normalized for the ischemic vasodilatory stimulus.

Results: In all individuals steps were reduced from PRE to SR (-82%;p<0.001), and then increased from SR to RT (+81%;p<0.01). Training adherence was 100% for both END and RES. Slope1 did not change from PRE to SR (p=0.53), and it was not affected by any RT intervention (all p>0.55). Time of ischemia and TSI_{min} were 411.5±99.8s vs. 491.2±88.2s;p<0.01 and 48.1±6.0% vs. 44.3±8.6%;p<0.05, respectively. $Slope2_{10}$ was reduced from PRE to SR (1.40±0.69%·s^{-1} vs. 1.00±0.29%·s^{-1};p<0.01). $Slope2_{10}$ partially recovered in END (1.03±0.51%·s^{-1};p<0.05) whereas it did not change after RES (1.01±0.34%·s^{-1} vs.1.26±0.74%·s^{-1};p>0.23).

Discussion: After 14 days of step reduction, microvascular responsiveness was impaired, suggesting that even a mild reduction of daily activity can induce alterations at endothelial level. Endurance training was an effective training strategy for recovering microvascular responsiveness within a short time after a period of inactivity, whereas resistance training was not.

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NIRS Sheds Light on the Heightened Metabolic Demands of Hypertrophy Training

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Introduction: Muscular adaptations to different resistance training (RT) protocols have been studied extensively. Recent research has focused on elucidating specific mechanisms that underpin adaptations such as hypertrophy and strength. This study will investigate the acute metabolic stress and mechanical tension during traditional hypertrophy (HYP) and strength (STR) RT protocols with the aim of improving RT program design and periodisation strategies.

Methods: Eight young resistance-trained men completed two experimental trials consisting of unilateral leg extension exercise (HYP: 10 reps x 4 sets, 70% 1RM, 1-min inter-set rest; and STR: 3 reps x 6 sets, 90% 1RM, 4-min inter-set rest). The *m.* vastus lateralis (VL) oxygenation (SmO_2; Near-infrared spectroscopy; NIRS) and *m.* rectus femoris (RF) muscle activation (electromyography; EMG) were measured during each trial. Maximum voluntary isometric contraction (MVIC) force was measured before and after each RT protocol. Femoral artery occlusion (250 mmHg; 5 min) was performed before training for SmO_2 signal calibration and subsequent analysis (Mileva et al., 2006). EMG root mean square (RMS) amplitude and median frequency (MDF) were normalised to the respective values during MVIC and statistically compared between the repetitions, sets and RT protocols using Spike2 software.

Results: VL SmO_2 was lower during the first (-108%) and last sets (-146%) of HYP compared to STR (Fig.1A). RF EMG RMS amplitude was higher during STR RT (Fig.1B). RF MDF decreased within the sets in both RT protocols but increased across the sets only in the STR.

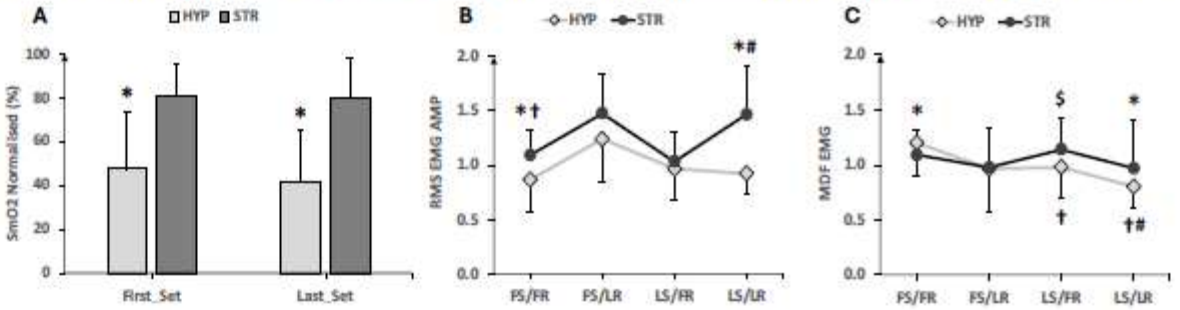

Figure 1. Changes (mean ± SD) across the hypertrophy (HYP) and strength (STR) resistance training (RT). **A.** *m.* vastus lateralis muscle oxygen saturation (SmO_2) during the first and last set of the RT. **B.** *m.* rectus femoris root mean square electromyography (EMG) amplitude (RMS AMP) and **(C)** median frequency (MDF) normalised to pre-exercise MVIC; first set / first rep (FS/FR), first set / last rep (FS/LR), last set/ first rep (LS/FR) and last set / last rep (LS/LR).

Discussion: HYP induced higher metabolic stress presented as heightened muscle deoxygenation. This is likely linked to longer time under mechanical tension and prolonged blood pooling (venous-arterial compression) during the sets combined with short inter-set rest periods (Tamaki et al., 1994). STR elicited higher muscular activation associated with the high-intensity protocol concomitant with lower metabolic stress.

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Negative impact of 14 days of steps reduction on muscle oxidative capacity in young participants

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Introduction: Inactivity negatively affects cardiorespiratory and muscle functions, decreasing exercise tolerance and dramatically increasing disease risks [1]. Previous data showed a time-dependent impairment of muscle oxidative capacity after severe inactivity (bed rest, BR) [2-4]. No changes in mitochondrial function were observed after 7 days of step reduction (SR) [5], a milder inactivity model. Therefore, we investigated the negative impact of 14 days of SR on muscle oxidative capacity estimated by near infrared spectroscopy in young participants. We hypothesized a longer period of SR could negatively affect muscle oxidative function.

Methods: Thirty (23±3 yr; 68±10 kg; 170±7 cm) participants followed a 14 days SR protocol. Before (T1) and after (T2) inactivity, whole-body maximal oxygen uptake (VO_{2max}) was measured during incremental cycling exercise. Vastus lateralis muscle oxygen uptake recovery rate constant (k) was calculated from tissue oxygen saturation index (TSI) changes during repeated transient occlusions performed immediately after leg-extension exercise. Time of ischemia-reperfusion for the occlusions was manipulated to maintain TSI in non-limiting O_2 availability condition [6]. Muscle biopsies were also collected and high resolution respirometry (HRR) was performed.

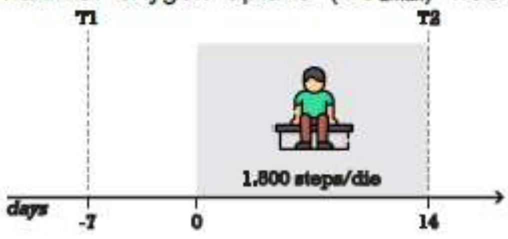

Figure 1: Overview of the protocol depicted on a timeline.

Results: SR resulted in an 82% reduction of participants' average daily steps. VO_{2max} was significantly reduced after SR (-4.4% from T1; p 0.022). *k* was significantly lower in T2 (2.1±0.6 min^{-1}) compared to T1 (2.3±0.6; p<0.01) as displayed in figure 2. HRR was not different after inactivity (p>0.05).

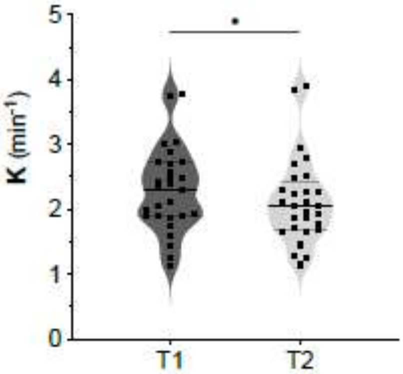

Figure 2: Violin plot describing vastus lateralis muscle oxygen uptake recovery rate constant (k) before (T1) and after (T2) 14 days of steps reduction in young participants.

Conclusions: Our data confirm that, after a mild reduction in daily activity for two weeks, whole body maximal oxygen uptake loss can be partially attribuited to limitations in oxygen flow at skeletal muscle level measured *in-vivo*. The absence of changes in maximally [ADP] stimulated mitochondrial respiration and other respirometric parameters after SR, which confirm previous data after 10 days bed rest interventions [4], raises interests towards other assessments of mitochondrial function, such as sensitivity of mitochondrial respiration to sub-maximal [ADP], and impairments upstream of mitochondria.

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Muscle microvascular responses to relative occlusive pressures

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Abstract: Blood flow restriction training has been proven to enhance muscle growth and strength. This study applied relative pressures of 40%, 60%, 80%, and 100% of limb occlusion pressure (LOP) on the right gastrocnemius, to characterize changes in O_2 availability at the microcirculatory level. Findings highlight that 40% of LOP may not completely occlude venous outflow from the muscle tissue, while 60% or 80% pressures induce maximal oxidized hemoglobin (HbO_2) accumulation due to total collapse of venous vessels. However, blood flow restriction must be maintained for 4-5 minutes to find significant differences in HbO_2 or reduced hemoglobin (Hb) concentrations during the occlusions.

Introduction: Training with external blood flow restriction (BFR) has been proven to enhance muscle growth and strength gains, probably through oxidative stress mechanisms. Guidelines suggest applying pressures between 40% and 80% of limb occlusion pressure (LOP) [1]. The present study aims to characterize real-time changes in O_2 availability at the microcirculatory level under different relative pressures.

Methods: LOP was determined for 23 participants (29.9 ± 5.6 years; 11 females). Four 5-minute vascular occlusion tests were randomly conducted on the right gastrocnemius, applying relative pressures of 40%, 60%, 80%, and 100% of LOP. Oxidized hemoglobin (HbO_2) and reduced hemoglobin (Hb) concentrations were measured using TR-NIRS.

Results: During partial occlusions (40%, 60%, 80% of LOP), HbO_2 values increased over time, showing differences between pressures. In the final (fourth and fifth) minutes, HbO_2 accumulation was significantly lower at 40% compared to 60% and 80% of LOP ($p<0.02$). In contrast, at 100% of LOP, HbO_2 decreased over time, particularly after the second minute ($p<0.002$). Regarding the Hb, values increased significantly minute by minute ($p<0.001$) across all pressures. Significant differences between relative pressures were observed only in the final minute of occlusion (40% < 60% < 80%; p=0.04)

Conclusions: 40% LOP pressure may not completely occlude venous outflow from the muscle tissue, while 60% or 80% have been demonstrated to elicit maximal HbO_2 accumulation due to total collapse of venous vessels. However, blood flow restriction must be sustained for 4-5 minutes to find differences in the HbO_2 or Hb concentrations among the applied pressures. The distinct responses in HbO_2 and Hb emphasize the importance of considering the LOP value and the time of occlusion when implementing a BFR protocol.

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(20)

NIRS evaluation of muscle oxidative capacity in neurological disorders

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Introduction: Skeletal muscle shows a high structural and functional heterogeneity and a high degree of plasticity [1]. Different physiological stimuli such as exercise training, disuse and ageing can rapidly change mitochondria in the skeletal muscle [2]. Similarly, several neurological diseases are characterized by impairments in skeletal muscle oxidative capacity due to a reduction in mitochondrial content as well as dysfunctions of the mitochondrial respiratory chain [3,4]. Near-infrared spectroscopy (NIRS) can non-invasively estimate muscle oxidative capacity in healthy individuals and disease population [5,6]. This study presents muscle oxidative capacity data obtained by NIRS in patients with different neurological diseases.

Methods: Thirty-six patients with neurological diseases were recruited: Friedreich's Ataxia (FA - n=18, age: 26±7 years, disease duration: 10.4±6.1 years, SARA scale: 17±6 points), Parkinson (PD - n=12, age: 60±8 years, disease duration: 3.7±2.0 years, UPDRS-III: 21±8 points), Cerebral Palsy (CP - n=6, age: 27±5 years, Physical Activity IPAQ: 1070±926 MET-min/week). Fifteen healthy young ($CTRL_Y$ - n=15, age: 27±4 years) and twelve elderly ($CTRL_E$ - n=12, age: 67±12 years) individuals were also recruited. Muscle oxidative capacity of gastrocnemius medialis (GM) was estimated by NIRS assessing muscle oxygen consumption recovery rate constant (*k*) from changes in tissue saturation index during intermittent occlusions of popliteal artery [7].

Results: Patients with neurological diseases showed the following *k* values: 1.24±0.51min^{-1} in FA, 1.49±0.38min^{-1} in PD and 1.01±0.11min^{-1} in CP. Healthy individuals revealed a *k* value of 2.49±0.80min^{-1} for $CTRL_Y$ and 1.94±0.41min^{-1} for $CTRL_E$.

Discussion: The present data demonstrated that NIRS technique is an effective non-invasive approach for detecting differences in muscle oxidative capacity between healthy individuals and patients with different neurological diseases.

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Changes in muscle optical proprieties during prolonged bed-rest and subsequent rehabilitation in young subjects via a time domain NIRS tissue oximeter

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Introduction: Near-infrared spectroscopy (NIRS) is a noninvasive methodology classically utilized to determine the concentration of light-absorbing chromophores in biological tissues [1]. The robustness of the tissue oxygenation/deoxygenation estimates obtained by classical continuous wave NIRS equipment is affected by changes in cutaneous and subcutaneous tissue characteristics (i.e. melanin, adipose tissue), muscle fiber architecture, capillary distribution and mitochondrial function [2,3]. Time domain (TD) NIRS oximetry, providing direct estimation of absorbance (μ_a), and reduced scattering (μ'_s), is becoming a more reliable tool to track changes in the matching between oxygen delivery and utilization occurring within the muscle [3,4]. Prolonged muscle disuse, classically simulated by bed-rest study, induces prominent changes in skeletal muscle structure, microvasculature and muscle oxidative capacity [5]. However, no data are present to describe the effects of bed-rest on optical characteristics of skeletal muscle. In this study TD-NIRS was used to monitor changes in muscle oximetry during prolonged bed-rest and subsequent rehabilitation in young subjects.

Methods: Nine (n=9) young participants (23 ± 4 years) underwent 21 days of bed-rest and subsequent 3 weeks of endurance-based rehabilitation. A TD-NIRS commercial tissue oximeter (NIRSBOX, PIONIRS srl, Italy) was employed to monitor the evolution of optical parameter (μ_a, μ'_s, DPF) and retrieve HHbMb, HbMbO2, HbMbtot and SmO2. Data (10-s duration, with an acquisition frequency of 10 Hz) were acquired on the vastus lateralis muscle of each subject in five consecutive sessions: pre-bed rest, after 9 days and after 20 days of bed rest, immediately post-bed rest, and post-rehabilitation. Statistical analysis was performed using one-way ANOVA for repeated measurement, with paired t-tests post-hoc comparison to identify significant differences (Holm-Bonferroni's corrected p-value < 0.05) between measurement points.

Results: After bed rest, a 15% increase of μ'_s values were registered, from pre-bed rest values of 11.5 ± 0.8 cm-1 (685 nm) and 10.6 ± 0.6 cm-1 (830 nm). Pre-bed rest values of DPF (5.1 ± 0.8 (685 nm) and 4.8 ± 0.7 (830 nm)) increased of 14% after 20 days of bed rest. Trends towards baseline conditions post-bed rest and after the retraining period were recorded. Related variations in skeletal muscle tissue oximetry estimates were also observed.

Conclusions: Bed rest significantly affected optical parameter (μ_a, μ'_s, DPF) of muscle tissue. TD-NIRS measurements should be taken into consideration to track changes in the tissue oxygenation/deoxygenation estimates after interventions affecting muscle optical characteristics.

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Evaluation of the Effects of Compression Garments on SmO_2 during Maximal Isometric Contractions

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Introduction:
Compression garments have gained popularity in the sports world as a means to enhance athletic performance and expedite recovery, given that fatigue is a major cause of exercise-induced muscle damage, compromising athletic performance and muscle recovery. This study investigates the effectiveness of upper body compression garments and their impact on muscle oxygen saturation (SmO_2), deoxygenation rate, and reoxygenation rate during maximal isometric contractions.

Methods:
Eight adult males, students enrolled in a master's degree program in Exercise Science, participated in the study, conducted under controlled conditions at the University of Salerno. The subjects performed maximal isometric contractions in three separate sessions, wearing compression garments (CG), traditional sportswear (noCG), and tight-fitting clothing without compressive effects to minimize the placebo effect (Placebo). In each experimental session, subjects performed three sets of isometric bicep curls with both arms, maintaining a 90° elbow flexion angle at 100% of maximal voluntary contraction for 10 seconds. Recovery between trials consisted of a 180-second passive rest period. SmO_2 was measured using the MOXY® monitor applied to the belly of the biceps brachii muscle. Deoxygenation and reoxygenation rates were observed under the three conditions.

Results:
The analysis of deoxygenation and reoxygenation rates was conducted through regression lines and subsequently assessed statistically using ANOVA to identify differences. No significant differences were found among the three conditions in both the deoxygenation and reoxygenation phases.

Discussion:
The results suggest that compression garments do not significantly influence deoxygenation and reoxygenation rates during maximal isometric contractions. These findings indicate that the effectiveness of compression garments may not be related to improvements in muscle oxygen saturation but could offer other benefits not measured in this study. Further research is needed to explore other potential advantages of compression garments, such as comfort, perceived effort, and overall recovery.

Promising Applications of Near-Infrared Spectroscopy: a 12-Week Supervised Exercise Programme for Patients with Peripheral Arterial Disease

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Introduction: Peripheral arterial disease (PAD) is a prevalent subtype of atherosclerotic cardiovascular diseases that affects 236.62 million people worldwide (1). With supervised exercise being considered the gold standard non-surgical treatment, the use of accurate technologies to monitor the internal load is essential. Near-infrared spectroscopy has been widely used as a technique for evaluating the behaviour of muscle oxygen saturation (SmO_2) and thus understand the dose-response locally (2). With most studies limiting themselves to characterising an acute evaluation session, this pilot study aimed to analyse the effect of 12-week supervised exercise programme on muscle response during a graded treadmill exercise test. **Methods**: Twelve adults with intermittent claudication were allocated to either supervised exercise training (SET; n=6; age=65.7±6.4yrs) or usual care (UC; n=6; age=64.2±4.2yrs). The SET comprised three sessions of cardiovascular and lower-extremity resistance training per week, with the load adjusted to the patient's individual response every 2-4 weeks, and the UC was advised to walk without specific recommendations. Pain-free walking distance (PFWD) and maximum walking distance (MWD) were registered. SmO_2 derived-parameters in *gastrocnemius medialis* of the most affected leg were calculated, specifically the average value during the first stage ($SmO_{2\,avg}$), the amplitude of deoxygenation ($SmO_{2\,deoxy}$), and the minimum value ($SmO_{2\,min}$). A repeated measures ANOVA was conducted to analyse the interaction 2 (group) x 2 (time), followed by Bonferroni's post hoc adjustment. **Results**: From baseline to 12-week, the SET increased their PFWD and MWD by 168.3±63.6m and 337.3±55.3m, respectively (mean, 108.6±38.6m to 276.9±159.1m [p=0.046] and 259.4±101.0m to 598.6±183.9m [p=0.002], accordingly), and no significant differences were observed in the UC in none of these variables. Even though no differences were identified in $SmO_{2\,deoxy}$ ($F(1,8)=1.66$; p=0.233; $n^2=0.04$) and in $SmO_{2\,min}$ ($F(1,8)=0.32$; p=0.589; $n^2=0.01$), the UC had a higher variation in $SmO_{2\,deoxy}$ compared to the SET after the 3-month (-8.8%, p=0.563 vs. -1.1%, p=1.000) and a decrease in $SmO_{2\,min}$ while it increased in SET (-0.7%, p=1.000 vs. 2.1%, p=1.000). The $SmO_{2\,avg}$ showed no changes. **Discussion**: The results revealed that in line with the improvements observed in PFWD and MWD, the balance between oxygen supply and consumption was more stable in SET, through lower deoxygenation and an increase in the minimum value reached, indicating a better peripheral adaptation. This insight could be a promising way of understanding the dynamics oxygenation and haemodynamics in muscle tissue and thus determine the optimal stimulus-response in PAD.

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O_2 diffusion limits oxidative capacity in young and middle-age, but not in elderly muscle

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Introduction: Aging leads to significant decrease in aerobic capacity, cardiovascular function (1), muscle strength and power, muscle mitochondrial oxidative capacity and capillarity (2). In older people, muscle fiber atrophy and loss of fiber oxidative capacity (3, 4) may counter effect muscle capillary rarefaction (4) and thereby protect O_2 diffusive flow into contracting muscle fibers by increasing the capillary supply region (5). We aimed to evaluate age-related differences in muscle oxidative capacity and O_2 diffusion *in vivo*.

Methods: Twelve young (YG; 26±5y), nine middle-aged (MID; 57±5y) and twelve old (OLD; 78±5y) individuals volunteered. They underwent an incremental cycle-ergometry to the limit of tolerance for peak oxygen uptake ($\dot{V}O_{2peak}$). In a different occasion vastus lateralis $\dot{V}O_2$ recovery rate constant (*k*) was measured by near-infrared spectroscopy during repeated transient occlusions after moderate exercise. Duration and timing of occlusions were manipulated to keep tissue saturation index (TSI) within 10% bounds of two different O_2 availability conditions: not-limiting (HIGH) and limiting (LOW) (6). *k*HIGH provides an estimate of muscle oxidative capacity, while the difference between *kHIGH* and *kLOW* (Δk) is inversely proportional to capillarity (a high Δk reveals O_2 diffusion limitation). One-way ANOVA with Tuckey's *post hoc* test was used to test differences among groups and one sample *t* test was used to test Δk difference from zero in each group. Linear regression was utilized to analyze correlation of $\dot{V}O_{2peak}$ with *k*HIGH and Δk.

Results: YG had greater $\dot{V}O_{2peak}$ than MID and OLD (35.7±8.2 vs. 30.1±5.6 and 24.2±4.4ml·min^{-1}·kg^{-1} respectively; $p<0.05$). *kHIGH* was greater in YG (2.97±0.55min^{-1}) compared to MID (2.09±0.63min^{-1}, $p<0.05$) and OLD (2.04±0.57min^{-1}, $p<0.01$), but MID and OLD were not different ($p=0.985$). Δk was significantly greater than zero ($p<0.05$) in YG (1.32±0.70min^{-1}) and MID (0.62±0.62min^{-1}), but not in OLD (0.02±0.65min^{-1}; $p=0.941$). Linear regression showed $\dot{V}O_{2peak}$ significantly correlated with both *k*HIGH ($r=0.40$; $p<0.05$) and Δk ($r=0.38$; $p<0.05$).

Discussion: Age-related differences in maximal aerobic capacity were related to skeletal muscle oxidative capacity and O_2 diffusion limitation. Unlike young and middle-aged muscle, diffusive capacity was not limiting to muscle oxidative capacity in the elderly. Thus, our data suggest age-related muscle adaptations help to protect muscle oxidative function in older aging. Future analyses on muscle samples to better characterize muscle structure and function will help to gain insights into age-related differences in limitations to intramuscular O_2 flow.

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Evaluation of muscle vascular function by time-domain near infrared spectroscopy

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Introduction: Physical exercise increases shear stress in the vasculature leading to a better vasodilator and constrictor responsiveness, reducing arterial stiffness and oxidative stress. Acute hemodynamic adaptations to exercise lead to changes in both endothelial and vascular smooth muscle cells functions. Regular exercise training improves nitric oxide (NO) availability. Monitoring muscle vascular function can be useful to evaluate the effectiveness of physical exercise at research or clinical level. **Aim:** We propose a method for the evaluation of muscular tissue's vessel stiffness, endothelial and vasomotor function, based on the frequencies of the oscillations of the hemodynamic parameters measured by fast Time Domain (TD) near infrared spectroscopy (NIRS). **Methods:** A fast TD NIRS acquisition (20 Hz acquisition rate, 4 cm interfiber distance, 690 nm and 830 nm)[1] on the tibialis anterioris muscle on a healthy subject was performed during 15 min at rest. TD NIRS data were analyzed using the solution of the photon diffusion equation for homogeneous media and the absolute values for oxy- (O2Hb) and deoxy- (HHb) hemoglobin concentrations were obtained from the absorption coefficients through the Beer's law. A blood volume pulse (BVP) sensor located on the same muscle and a respiration band (RESP) were also employed to record physiological signals. After a 3rd order polynomial detrending, the power spectral density (PSD) for all the parameters were calculated exploiting a Matlab script (Welch algorithm, 7-min Hamming window, 0.5 overlapping factor). The mean PSD in six frequency bands related to peculiar muscular activities was derived:[2] Endothelial (VI), NO-related Endothelial (V), Neurogenic (IV), Myogenic (III), Respiratory (II) and Cardiac (I). **Results:** Most of the PSD power for the TD NIRS parameters is contained in the endothelial bands, in particular in band VI for HHb and band VI and V for O2Hb (Fig.1a). The PSD for BVP (Fig.1c) is similar to O2Hb and HHb, while a strong contribution to band II is found in the PSD for RESP, as expected (Fig.1b). **Conclusion**: Fast TD NIRS measures can be used to determine the PSD of the hemodynamic parameters, gaining insight on muscle vascular functions.

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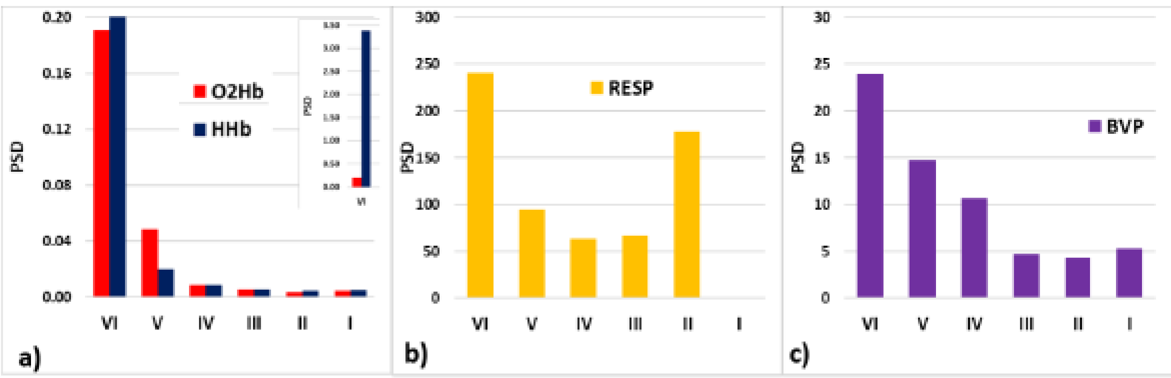

Fig1. Mean PSD (a.u.), in the six frequency bands for O2Hb and HHb (a), respiration sensor (RESP) (b) and Blood Volume Pulse Sensor (BVP) (c). In panel (a) it is shown a zoom for PSD<0.2; in the inset the total PSD for band VI, which has a peak > 3 for HHb.

Noninvasive assessment of muscle oxidative metabolism and microvascular function in response to glucose ingestion in individuals with obesity *vs* lean controls

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Introduction: Obesity is associated with metabolic resistance and impairments in vascular function that can be enlightened with an Oral Glucose Tolerance Test (OGTT), and affects upper and lower limbs districts differently. **Purpose:** full characterization of both metabolic and microvascular responses to VOT and OGTT in the upper and lower limbs, in obese and lean individuals.

Methods: 10 lean (Ctrl, 5 females, 67±6 years, 24±2 BMI) and 10 mild obese (Ob, 5 females, 68±3 years, 31±2 BMI) individuals underwent 5 vascular occlusion tests (VOT): pre-intervention (Pre), 30, 60, 90, and 120min during OGTT. Oxygen saturation (StO_2) was measured with Near-Infrared spectroscopy (NIRS) at the flexor digitorum longus (Ob_{arm} and $Ctrl_{arm}$) and tybialis muscles (Ob_{leg} and $Ctrl_{leg}$) at each VOT. Muscle oxidative metabolism and microvascular responsiveness were determined respectively as the StO_2 desaturation slope and StO_2 reperfusion slope during cuff inflation and deflation.

Results: Pre-values show no differences in oxidative metabolism and microvascular responsiveness among groups and between lower and upper limbs. However, OGTT significantly blunted metabolic and microvascular responses in Ob_{arm} vs $Ctrl_{arm}$, at 30min (desaturation slope, Ob_{arm} = 0.06 ± 0.02%·s^{-1}, $Ctrl_{arm}$ = 0.10 ± 0.03%·s^{-1}; reperfusion slope, Ob_{arm} = 1.09 ± 0.40%·s^{-1}, $Ctrl_{arm}$ = 1.45 ± 0.56%·s^{-1}) at 90min (desaturation slope, Obarm = 0.07 ± 0.02%·s^{-1}, $Ctrl_{arm}$ = 0.11 ± 0.03%·s^{-1}; reperfusion slope, Ob_{arm} = 1.15 ± 0.37%·s^{-1}, $Ctrl_{arm}$ = 1.45 ± 0.45%·s^{-1}) and at 120min (desaturation slope, Ob_{arm} = 0.07 ± 0.02%·s^{-1}, $Ctrl_{arm}$ = 0.11 ± 0.02%·s^{-1}; reperfusion slope, Ob_{arm} = 1.15 ± 0.36%·s^{-1}, $Ctrl_{arm}$ = 1.48 ± 0.43%·s^{-1}) while at 60min only the metabolic response was significantly lower (desaturation slope, Ob_{arm} = 0.06 ± 0.02%·s^{-1}, $Ctrl_{arm}$ = 0.10 ± 0.03%·s^{-1}) (p<0.05 for all comparisons). No significant differences were found between Ob_{leg} and $Ctrl_{leg}$. Furthermore, there was a significant increase in $Ctrl_{arm}$ metabolic and microvascular responses, at respectively 120min and 90min, when compared with their Pre values (Pre, desaturation slope 0.09 ± 0.03%%·s^{-1}; reperfusion slope 1.31± 0.49%·s^{-1}) (p<0.05 for all comparisons) while no effect of time was found on either Ob_{arm}, $Ctrl_{leg}$ and Ob_{leg}.

Discussion: Our study demonstrated differences in muscle oxidative metabolism and microvascular reactivity in the upper limb between lean and obese individuals. Furthermore, limb-related differences in response to glucose ingestion were found only in lean individuals.

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Near-infrared spectroscopy as a tool to identify mitochondrial and microvascular biomarkers of aging

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Introduction: A decline in skeletal muscle function is thought to be a key factor contributing to the decreased functional capacity and quality of life associated with aging. Although the exact mechanism is not fully understood, impaired mitochondrial and microvascular function are thought to play a major role. Near-infrared spectroscopy (NIRS) can be a promising tool for finding biomarkers that can characterize the aging process and evaluate exercise programs that aim to preserve muscle function at older age. However, a complicating factor in determining the physiological mechanisms of aging is the fact that aging typically coincides with a decline in physical fitness due to lifestyle changes. In addition, changes in body composition that are known to occur with aging, can further complicate the quest in finding biomarkers of aging using NIRS. The main purpose of this study was to investigate possible aging effects on microvascular and mitochondrial function in normal aging adults. In addition, the relationship between microvascular and mitochondrial function with performance variables, as an indication of physical fitness, and body composition variables, as confounding variables for the NIRS method were investigated.

Methods: Eighteen healthy young adults (HY: 25.8 ± 2.3 yrs) and eighteen healthy older adults (HO: 69.8 ± 6.0 yrs) adults participated in this study. Mitochondrial and microvascular function in arm and leg were measured using NIRS during and following a vascular occlusion. Physical fitness was evaluated through graded cycling and handgrip tests, while body composition was measured by skinfold thickness and bioimpedance analysis. Data were analyzed using repeated measures ANOVA and multiple regression analysis.

Results: Age alone did not significantly impact desaturation and resaturation responses provoked by vascular occlusion, as no group differences were found in muscle oxygen consumption, reperfusion rate, maximum desaturation during occlusion and peak resaturation during hyperemia. However, an interaction effect suggested age-related differences in NIRS responses between forearm and leg muscles. Multiple linear regression showed that age emerged as a moderate predictor of mVO_2 in forearm muscles, accounting for 15% of variation, independent of other predictors in the model. This was not the case for the leg muscles where not age, but skinfold thickness was found to be a strong predictor for mVO_2, explaining 38% of the variation.

Discussion: This study indicates that aging does not uniformly affect muscle function as measured by NIRS. Although age-related changes in muscle function can be detected during vascular occlusion at rest, these changes can be easily masked by differences in training status, body composition (specifically skinfold thickness), or variations between muscles. Relying solely on a general measure of body composition (such as BMI) is insufficient due to the uneven distribution of fat throughout the body and the changes that occur due to aging. Given the confounding effects of physical fitness and local adipose tissue thickness, researchers should carefully report information about both factors. Addressing these complexities is crucial for understanding the true impact of aging on muscle function measured by NIRS.

An Investigation into the Relevance of Limb Dominance on Skeletal Muscle Near-Infrared Spectroscopy Measurements

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Introduction: Skeletal muscle oxidative capacity and microvascular function decline with ageing and many diseases. Near-infrared spectroscopy (NIRS) provides a non-invasive technique which, when combined with vessel occlusion, assesses oxidative capacity and microvascular function directly from the skeletal muscle. However, measurements can be highly variable and the effect of limb dominance on these NIRS measurements has received limited exploration.

Aims: To investigate the difference in the oxidative capacity and microvascular function between the dominant and non-dominant leg in a healthy adult population using NIRS.

Methods: Healthy individuals were recruited from a university student population. In all participants, NIRS was used to measure changes in oxygenated and deoxygenated haemoglobin during arterial occlusions at rest and post-exercise as well as during and after a 5-minute arterial occlusion. Measurements were performed on the left and right gastrocnemius. Leg dominance was self-reported. Local resting oxygen consumption (musVO_2), recovery in oxygen consumption after exercise (time constant) and post-occlusive reactive hyperaemia (PORH) were quantified using custom-written scripts (MATLAB® 2022a, The MathWorks, Inc, USA). A longer time constant indicated a reduced oxidative capacity. A longer PORH indicated poorer microvascular function. Descriptive statistics were presented as median (interquartile range) or proportion (percentage). Non-parametric testing due to small sample size and skewed distribution was used for analysis (significance level $p<0.05$).

Results: In total, 14 participants were recruited (8 [57%] men, median age 21 [20-21] years). There was a statistically significant increase in the time constant in the non-dominant leg (42.0s vs 77.4s, $p<0.001$). There was no statistically significant difference in the other parameters. There was a statistically significant strong positive correlation between the dominant and non-dominant leg for resting musVO_2 (r_s 0.732, $p<0.003$). There was weak positive correlation between dominant and non-dominant legs for the time constant (r_s 0.429, $p=0.126$) and the time to 95% PORH (r_s 0.095, $p=0.747$).

Conclusions: There is a persistent difference in oxidative capacity between the dominant and non-dominant leg. This will need to be accounted for in the assessment of clinical conditions and fitness levels.

Greater vastus lateralis extraction kinetics during moderate-intensity supine cycling in men compared to women

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The present study investigated sex differences in spleen emptying, red blood cell release and skeletal muscle bioenergetics during supine step-transition cycling at moderate intensity. We hypothesized that the reduction in splenic volume and subsequent manipulations of O_2 delivery by blood expelled from the spleen are not sex-specific, whereas women are characterized by faster $\dot{V}O_{2p}$ and NIRS-derived ΔHHb kinetics of the *vastus lateralis* (VL) compared to men. This difference is attributed to women having a greater oxidative capacity in the mitochondria of their VL muscle (Cardinale et al., 2018) and a higher capillary density in this muscle compared to men (Roepstorff et al., 2006). Eight men (age=23±4) and seven women (age=25±3) completed three laboratory assessments, including a detailed medical examination, a supine $\dot{V}O_2$ max. test, and three step-transitions from 20 W to a moderately intense power output corresponding to $\dot{V}O_2$ at 90% gas exchange threshold. During these step-transitions, pulmonary $\dot{V}O_{2p}$ and VL oxygenation (via NIRS) were measured continuously. In parallel, ultrasound measurements of the spleen were performed every minute (Zubac et al., 2022). Blood samples were taken before and immediately after step-transition cycling. Mean $\dot{V}O_2$ max. and baseline Hb levels were higher in men compared to women (44.8±6.2 vs 37.2±3.3 $mL \cdot min^{-1} \cdot kg^{-1}$ and 148±9 vs 127±5 g/L, respectively). In response to moderate-intensity cycling, spleen volume decreased significantly (by ~35% and returned to baseline values after 20 minutes, with both groups following a similar pattern). Hemoglobin concentration increased by ~3% equally in both sexes after completion of exercise. No differences were observed between the two groups in the phase II time-constant ($\tau\dot{V}O2p$) during moderate-intensity step-transitions (43 vs 47 sec., p=0.44). Time-course changes in ΔHHb showed differences between groups in the amplitude of VL deoxygenation, with the amplitude being ~35% (5.6 vs 19.1 μm, p=0.001) greater in males. The time delay and τ-time constant of the ΔHHb signal were similar between groups. Interestingly, a slower O_2 extraction from the superficial part of the VL in women was unexpected. Possibly, this is related to acute changes in perfusion pressure or regional differences in muscle fiber composition, size, and type. For instance, greater activation of type II fibers was observed in the supine model compared to classical cycling (Goulding et al., 2021), resulting in a higher O_2 cost for muscle contraction. Still, these findings require further confirmation.

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Sex differences in oxygenation of the intercostal and vastus lateralis muscles during incremental exercise

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Introduction: Physiological sex–related differences during dynamic physical exercise have been a subject of recent interest. A primary difference is the greater oxygen consumption of the respiratory muscles (VO_2–RMs), such as the *m.intercostales*, in women (13.8 vs 9.4% in men), requiring higher lung ventilation (VE) in response to metabolic demands. The increase of VO_2–RMs requires increased blood flow to the respiratory muscles, which may restrict nutrient and oxygen supply to peripheral exercising muscles, thereby limiting the continued performance of exercise. This study aimed to evaluate the sex differences of SmO2 in *m.intercostales* and *m.vastus lateralis* during an incremental cycle test until exhaustion and to analyze the changes in SmO_2 in relative to ventilatory variables, peripheral workload, and aerobic capacity (VO_2–*peak*)

Methods: Twenty–five (12 women) healthy and physically active participants were evaluated during an incremental test with a cycle ergometer, while ventilatory variables (VE, tidal volume (Vt), and respiratory rate (RR)) were acquired through the *breath-by-breath* method. SmO_2 was acquired using the MOXY™ devices on the *m.intercostales* and *m.vastus lateralis*.

Results: A two-way ANOVA (sex × time) indicated that women showed a greater significant decrease of SmO_2–*m.intercostales*, and men showed a greater significant decrease of SmO_2–*m.vastus lateralis*. Additionally, women reached a higher level of SmO_2–*m.intercostales* normalized to VE ($L \cdot min^{-1}$) (p<0.001), whereas men had a higher level of SmO_2–*m.vastus lateralis* normalized to peak workload–to–weight ($watts \cdot kg^{-1}$, PtW) (p=0.049), as confirmed by Student's t-test.

Discussion: During an incremental physical exercise, women experienced a greater cost of breathing, reflected by greater deoxygenation of the respiratory muscles, whereas men had a higher peripheral load, indicated by greater deoxygenation of the locomotor muscles.

Oxygenation of respiratory and locomotor muscles during the cardiopulmonary exercise testing in patients with Fontan circulation: case series

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Introduction: During a maximum incremental exercise patients with Fontan circulation (PFC) show cardiac limitation reducing aerobic exercise capacity (VO_2–peak). Cardiovascular rehabilitation (CR) reverses this deconditioning by increasing cardiac output and arteriovenous oxygen difference, aspects that can be evaluated by invasive methods and analyzing the exhaled gases. Non–invasive assessment of muscle oxygen saturation (SmO_2) is a novel method for recording local oxygen levels. By this technology, it is possible to evaluate the muscle limitation to exercise. In PFC, that limitation could be attributed to higher contractions of respiratory (ventilatory changes) and/ or locomotor muscles (peripheral load). The aim of this study was to evaluate in PFC the changes at SmO_2 of respiratory and locomotor muscles during a maximum and incremental exercise protocol (cardiopulmonary exercise testing, CPET).

Methods: Six PFC (5 men; 13.8 ± 2.9 years; 158 ± 9 cm; 49.8 ± 13.3 kg) were assessed during the VO_2–peak test (23.0±4.5 $mL \cdot kg^{-1} \cdot min^{-1}$) by cycloergospirometry synchronously with SmO_2 at respiratory (SmO_2–*m.intercostales*) and locomotor (SmO_2–*m.vastus laterallis*) muscles by *near–infrared spectroscopy (NIRS)*.

Results: SmO_2–*m.intercostales* decreased from 60% of VO_2–peak ($p<0.05$), while SmO_2–*m.vastus laterallis* did not change. Minute ventilation (VE) increased progressively, showing changes to rest at 60% of VO_2–peak ($p<0.05$). The higher deoxygenation of SmO_2–*m.intercostales* (ΔSmO_2) correlated to maximum changes of lung ventilation (ΔVE) (rho=0.80; p=0.05).

Discussion: During an incremental and maximum exercise protocol, patients with Fontan circulation have more work at respiratory than locomotor muscles. Changes in VE are direct associated with greater extraction of oxygen at respiratory muscles, reinforcing the incorporation of respiratory muscle training in cardiovascular rehabilitation.

The contribution of muscle oxygenation to resistance training

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Introduction: Hypertrophy training results in increased muscle size through enhanced protein synthesis, which is attributed to mechanical and metabolic stress (2). The examination of muscle oxygenation patterns identifying muscle occlusion generating hypoxic sequences can help prescribe an optimal intensity for resistance training, aiming to maximize hypertrophy and muscle performance gains (1). Therefore, the aim of this study was to propose an individualized approach to optimise resistance training outcomes for hypertrophy development. It was hypothesised that the establishment of an optimal intensity for hypertrophy using a near-infrared spectroscopy (NIRS) sensor would allow for the customisation of workloads in order to maximise the effects of resistance training on force production on one-repetition maximum (1RM) in squat and CrossFit performance.

Methods: A total of 12 CrossFit practitioners (12 males) aged between 20 and 34 years were randomly assigned to two groups (the "moxy group" and the "control group"). All participants had a minimum of two years of training experience and engaged in five training sessions per week. For a period of eight weeks, both groups engaged in two back squat sessions per week, with four sets per session. The training load was balanced between the two groups for each training session. The moxy group employed a protocol utilising the NIRS sensor to ascertain the optimal work intensity, based on the occurrence of arterial occlusion, as determined by linear regression (total haemoglobin and muscle O2 saturation) during a progressive isometric test, expressed as a percentage of 1RM in the back squat. The control group was required to perform a workload corresponding to 75% of their one-repetition maximum (1RM). The NIRS sensor (Moxy) was positioned on the right vastus lateralis (VL) muscle.

Results: A significant percentage of change was observed in terms of 1RM gains in squat (moxy = +4.26%, Hedge's g = 0.4 vs. control = +3.1%, Hedge's g = 0.6) and FE (moxy = +12.1%, Hedge's g = 0.9 vs. control = +7.8%, Hedge's g = 0.3), with the moxy group demonstrating greater gains than the control group.

Conclusion: Exploiting variations in muscle oxygenation variables during resistance training through the application of a NIRS protocol including the determination of occlusion thresholds, appears to promote performance gains in terms of 1RM and CrossFit performance. These preliminary findings require further confirmation through the assessment of anatomical and physiological cross-sectional areas of the active muscles.

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The relationship between muscle oxygen saturation breakpoints and ventilatory thresholds in triathletes

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Introduction: To optimize endurance training, individualized training zones should be determined based on physiological thresholds. NIRS technology has made it possible to non-invasively assess muscle oxygen saturation during physical exercise. Four phases of muscle oxygenation dynamics during incremental exercise have been documented (Bhambhani, 2004). This predictive behaviour has led to the comparison of ventilatory thresholds (VT) with SmO_2 thresholds (BP). The aim of the study was: to evaluate the relationship between ventilatory thresholds (VT_1 and VT_2) and SmO_2 thresholds (BP_1 and BP_2) in triathletes during a running incremental test. **Methods:** Twelve national level triathletes were assessed during a VAM EVAL test. Breath by breath gas exchange was recorded using a portable gas analyser, SmO_2 was measured using a continuous wave NIRS device placed on the right vastus lateralis, and heart rate was recorded using a Polar H10 attached to a chest strap. BP were determined using a piecewise double linear regression model because the double-linear model provided a superior fit in over 90% of the cases (Murias et al., 2013). **Results:** In seven subjects SmO_2 described a monotonic behaviour while in five subjects a parabolic behaviour was detected. BP and VT did not show significant differences when compared based on HR (VT_1 165.2 ± 14.9 bpm; BP_1 164.8 ± 19.9 bpm p= 0.925 and VT_2 168.8 ± 19.4 bpm; BP_2 171 ± 14.4 bpm p= 0.439), VO_2 (VT_1 51.3 ± 6.3 $ml \cdot kg^{-1} \cdot min^{-1}$; BP_1 52.1 ± 8.1 $ml \cdot kg^{-1} \cdot min^{-1}$ p= 0.636 and VT_2 58.1 ± 6.3 $ml \cdot kg^{-1} \cdot min^{-1}$; BP_2 59.1 ± 5.6 $ml \cdot kg^{-1} \cdot min^{-1}$ p= 0.327), velocity (VT_1 15.1 ± 1.1 $km \cdot h^{-1}$; BP_1 14.8 ± 1.6 $km \cdot h^{-1}$ p= 0.541 and VT_2 17.9 ± 0.6 $km \cdot h^{-1}$; BP_2 18.0 ± 1 $km \cdot h^{-1}$ p= 0.718) and calibrated SmO_2 (VT_1 47.8 ± 22.9 %; BP_1 54.2 ± 17.3 % p= 0.210 and VT_2 12.3 ± 19.4 %; BP_2 10.3 ± 16 % p= 0.480). The results of the Bland Altman plots for the first threshold displayed a very low mean error but a wide 95%CI while for the second threshold, the low mean error was coupled with a much narrower 95%. The multiple linear regression analysis displayed the most suitable equations to predict the VT_1 and VT_2 from the BP_1 data (HR, velocity and SmO_2). Our results indicated the following prediction percentage: 83% SmO_2 VT_1 and 78% HR VT_2. The relevant equations are shown:

$$SmO_2\ VT_1\ (\%) = -90.213 + (1.099 * SmO_2\ BP_1) + (5.408 * velocity\ BP_1)$$

$$HR\ VT_2\ (bpm) = 100.180 + (0.921 * HR\ BP_1) - (4.859 * velocity\ BP_1)$$

Discussion: In some subjects BP and VT occur simultaneously but in other subjects the difference between BP and VT was substantial. This high level of heterogeneity could be due to inter-individual differences in the distribution of different muscle fibre types and muscle activation patterns (Boone et al., 2016). The findings suggest that SmO_2 thresholds identified by NIRS can reliably predict ventilatory thresholds in highly trained athletes despite the heterogeneity of individual muscle oxygen saturation response as long as heart rate, velocity and SmO_2 data associated with BP_1 are available, supporting the use of NIRS in non-invasive monitoring of exercise physiology. This approach holds potential for optimising performance assessment and training regimens in high-level athletes.

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Exploring the role of muscle oxygen saturation during high intensity interval training in triathletes

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Introduction: Currently, most athletes monitor their running training sessions with pace, power, and heart rate (HR). However, these measurements don't quantify specifically how muscles respond to physical exercise. NIRS technology has made it possible to non-invasively assess muscle oxygen saturation (SmO_2) during physical exercise, and several authors confirm its use as a metabolic biomarker to measure acute and chronic responses to training (Perrey & Ferrari, 2018). This novel technology has led to research into the role of muscle oxygen saturation in the control of training. The aim of the study was: to evaluate the use of different physiological parameters such as HR, SmO2, THb and oxygen consumption (VO2) to control high-intensity interval training in triathletes. **Methods:** Fifteen national level triathletes completed a VAM EVAL test to determine maximal aerobic speed (MAS) and seven days later, performed a high intensity interval training (Figure 1). Breath by breath gas exchange was recorded using a portable gas analyser (Cosmed K5), HR was measured using a chest band (Polar H10) and SmO_2 was measured using a continuous wave NIRS device placed in the right vastus lateralis (Moxy). Mean and standard deviation (SD) was used to describe the variables. A one-way analysis of variance (ANOVA) was used to test differences between different sets, for the work phase the averages of the last 10 s of each series were taken, and for the recovery phase, we took the averages from the 50 s to 60 s, 80s to 90 s and 110 s to 120 s. **Results:** Table 1 presents the mean values for SmO_2, VO_2, HR, lactate (La) and total hemoglobin index (THb) during the high intensity interval training (n=15). **Discussion:** The main finding of this study is that HR is a more precise physiological parameter than SmO_2 in monitoring fatigue during the recovery phases of interval training at 100% MAS. The causes of athlete fatigue are diverse: on one hand, during the rest period between sets, heart rate recovery decreases, which could be due to reduced parasympathetic cardiac reactivation with the accumulation of sets (Coote, 2009). On the other hand, during the work phase, the reduction in VO_2 from set to set could be due to pulmonary limitation caused by the increase in cardiac output resulting from the increase in heart rate from set to set, which could reduce the time available to saturate the blood with O_2 before it leaves the pulmonary capillary (Bassett & Howley, 2000). This explanation is consistent with the behavior of THb, which increases throughout the series.

Table 1. Values during high intensity interval training.

	First set		Penultimate set		Last set
N_SmO₂_W(%)	9,25 ± 21,84		8,75 ± 21,71		5,40 ± 20,55
N_SmO₂_R50-60(%)	53,87 ± 12,14		55,07 ± 11,27		55,00 ± 20,82
N_SmO₂_R80-90(%)	59,87 ± 10,64		57,33 ± 12,61		60,60 ± 15,09
N_SmO₂_R110-120(%)	63,20 ± 11,44		60,80 ± 11,03		64,40 ± 11,84
HR_W(bpm)	177,13 ± 14,62		179,53 ± 13,83		183,49 ± 14,02
HR_R50-60(bpm)	130,92 ± 19,64	b	137,81 ± 17,08		145,39 ± 22,89
HR_R80-90(bpm)	120,50 ± 22,90		126,74 ± 20,22		126,97 ± 22,86
HR_R110-120(bpm)	119,92 ± 21,43		126,40 ± 18,63		120,00 ± 20,03
VO₂_W(ml·min⁻¹·kg⁻¹)	60,14 ± 7,59		59,67 ± 7,92		58,00 ± 7,63
VO₂_R50-60 (ml·min⁻¹·kg⁻¹)	23,49 ± 4,67		23,88 ± 4,82		24,57 ± 5,49
VO₂_R80-90(ml·min⁻¹·kg⁻¹)	18,98 ± 3,62		19,68 ± 4,24		18,76 ± 5,29
VO₂_R110-120(ml·min⁻¹·kg⁻¹)	18,68 ± 3,07		18,71 ± 3,39		16,62 ± 5,63
Thb_W	12,44 ± 0,36	b	12,46 ± 0,34		12,50 ± 0,32
La_W(mmol·L⁻¹)	7,90 ± 1,63	a b	9,47 ± 1,71	b	12,29 ± 2,19

Note: Values are mean ± SD. Level of significance is P< 0.05. Letters (a, b) indicate a significant difference between sets compared to penultimate set and last set, respectively. Normalized (N), work (W), recovery (R).

Figure 1. High intensity interval training protocol

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Changes in oxidative metabolism basal parameters in vastus lateralis for middle-aged and old populations in a 12 months period

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Introduction: The "Trajector-AGE" project (PRIN2020-2020477RW5) focuses on the study of neuromuscular decline in middle-aged (55–60yrs, MA) and old (75-80yrs, OLD) subjects with a multimodal approach to give a comprehensive characterization of the muscular status and to trace trajectories of aging during months. In this preliminary work, we assess the capability of time domain (TD) NIRS, to detect differences, if any, in the basal condition of the vastus lateralis muscle during a 12 months period in these two critical phases of aging. **Methods:** A total of 89 healthy subjects were evaluated at a first (T0), a second (T1, n=67) and a third (T2, n= 26) time point (TP, 6 month interval between each TP). The number of subjects evaluated at T1 and T2 is lower, since the acquisitions are still running. The acquisitions were performed with subjects sat on a cycle ergometer, with 0.8 Hz sampling rate and 2.5 cm source-detection separation on the vastus lateralis (proximal).[1] The adipose tissue thickness (ATT) was also measured with a skin folder caliper. Data were analyzed using the solution of the photon diffusion equation for homogeneous media. The absolute values for: absorption (μ_a) and scattering (μ_s') coefficient, oxy- (HbO_2) deoxy- (HHb) and total- (tHb) hemoglobin and oxygen saturation (SO_2), were computed as the average among 10-s of acquisitions. Boxplots for the previous parameters were created and statistical significance among groups were tested with two-sample Kolmogorov-Smirnov test. **Results:** In Fig 1, the boxplots for SO_2, at the 3 TPs are shown for MA (a,b) female (F,a) and male (M,b), and OLD (c,d) F (c) and M (d). The only significant difference for these parameters was found between F and M of the same age ($p=0.02$ for OLD; $p<10^{-4}$ for MA).The differences between MA and OLD could be due to the differences in ATT ($p=0.01$ for OLD and $p<10^{-7}$ for MA).

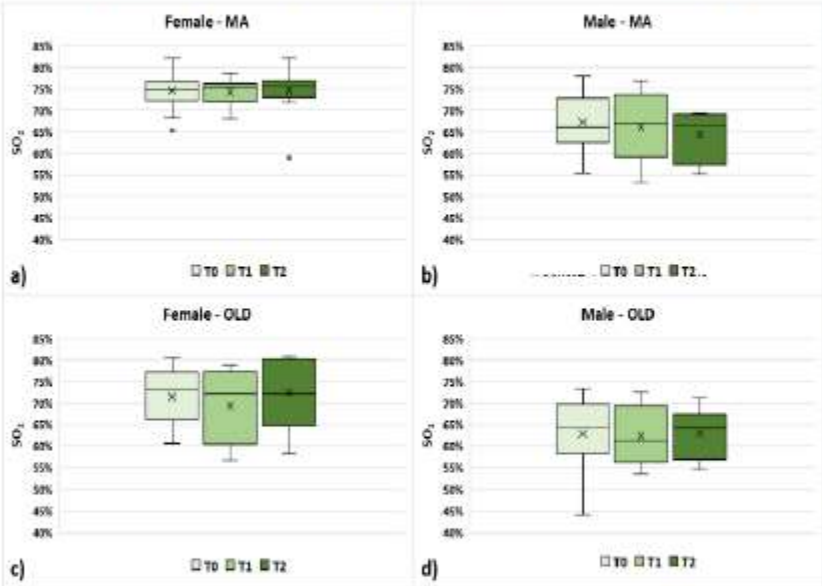

Fig. 1 Absolute value for SO_2 calculated at the 3 TP for MA (a, b) and OLD (c, d) female (a,c) and male (b,d).

Conclusion: absolute values for basal SO_2 seems not to present significant differences between MA and OLD population.

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Muscle oxygenation during different blood volume alteration exercise protocols: application to the hamstrings

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Introduction: A method known as low intensity strength training without relaxation may result in a reduction in blood flow during dynamic exercise (2). However, no study has investigated the variations in blood volume during dynamic movement sequences without rest. A significant proportion of studies have measured the oxygenation of the quadriceps, but only a small number have examined the hamstrings (1). This muscle group is susceptible to damage during rapid changes in contraction modes, such as from eccentric (Ecc) to concentric (Con). Thus, this study aimed to investigate the oxygenation responses of the hamstrings during different blood volume alteration exercise protocols combining alternating regimens of Ecc and Con contractions without resting on an isokinetic dynamometer.

Methods. In protocol 1, a sample of 12 healthy participants carried out 3 exercise modes (30% of peak Ecc/Con torque without rest, 70% of peak Ecc/Con torque with or without rest). In protocol 2, a second sample of 12 healthy adult participants performed two equivalent bouts of dynamic exercises (30% of peak Ecc/Con torque without rest versus 30% of peak Ecc/Con torque with blood flow restriction (BFR 60% limb occlusion pressure with Made-Up®), where the rest phase was equivalent to the working time between each contraction). Variations in blood volume and muscle oxygenation were quantified by near-infrared spectroscopy (NIRS Portamon, Artinis) positioned at the mid-level of the hamstrings.

Results: In protocol 1, the results showed a significantly less decrease in blood volume ($p = 0.026$) for the 70% intensity with versus without rest. Additionally, muscle deoxygenation was significantly lower for the 70% condition compared to the other two resting modalities (70% and 30%, $p = 0.006$ and $p = 0.011$, respectively). In protocol 2, the mean values of total haemoglobin and deoxyhaemoglobin varied equally (TOST procedure for two one-sided tests) between contractions without rest and those with BFR.

Conclusion: The results collectively indicate a reduction in blood flow and an increase in muscle deoxygenation during the absence of a resting phase, which ultimately leads to a state of ischemia. The development of low-intensity muscle strengthening programs without a rest phase may be beneficial in the context of post-injury muscle healing.

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Muscle Hemodynamic and Metabolic Response to Blood Flow Restriction with Exercise

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Abstract: Hemodynamic response to blood flow restriction (BFR) with and without exercise on calf muscle is studied with hybrid diffuse optical measurements.

Introduction: BFR training involves applying sub-systolic cuff pressure at the proximal end of a limb to restrict blood flow. It is increasingly being adopted to enhance muscle hypertrophy, especially after an injury. However, BFR leads to heterogeneous responses and, sometimes, poses some safety concerns. To better understand and propose evidence based BFR methods, it is important to study the changes in muscle hemodynamics and oxygen metabolism under BFR. To that end, we have recruited 23 (protocol 1, no exercise) and 5 (protocol 2, with exercise) healthy volunteers to study the hemodynamic response on calf muscle with and without exercise at different BFR pressures respectively and studied: (i) repeatability and variability of BFR, (ii) dependence on the duration and order of pressure increase, and (iii) the effect of exercise on different parameters.

Protocol: The protocol 1 consists of 2min baseline, 5min occlusion and 3min release periods while protocol 2 consists of isometric ankle plantar flexion exercise (4 sets of 30-15-15-15 reps of 2s contraction and 2s relaxation with 30s of rest between each set of reps) in the supine position at the BFR pressures 0%, 40%, 80% of limb occlusion pressure (LOP). The LOP of each volunteer is estimated by applying incremental cuff pressures on proximal part of the thigh and concurrent monitoring of popliteal artery with Doppler ultrasound. The exercise is performed at 30% of maximal voluntary contraction (MVC). The hemodynamic data was measured with hybrid diffuse correlation spectroscopy (DCS, ICFO custom-made) and time resolved spectroscopy (NIRSBOX, PIONIRS SL, Italy) systems. The electromyography (EMG) signals on the calf muscles and force on the toes during the exercise are measured with a standard EMG (SX230), and a dynamometer logged at 1KHz (P3X8) from Biometrics Ltd, UK.

Results: Large variability in the hemodynamic data during occlusion and hyperemic response (HR) are observed in protocol 1, in Fig 1(a) and 1(b). Fig. 1(c) and 1(d) shows protocol 2 data for one subject only suggesting that under low BFR pressures (0%, 40% LOP) with exercise, the blood flow index (BFI) stay elevated while tissue oxygen saturation (StO_2) heads to recovery during the rest periods. At higher BFR level (80% LOP), BFI is less elevated while StO_2 does not recover, suggesting greater hypoxia in the calf muscle. The information from protocols 1 and 2 can be used to safely optimize the BFR regimens.

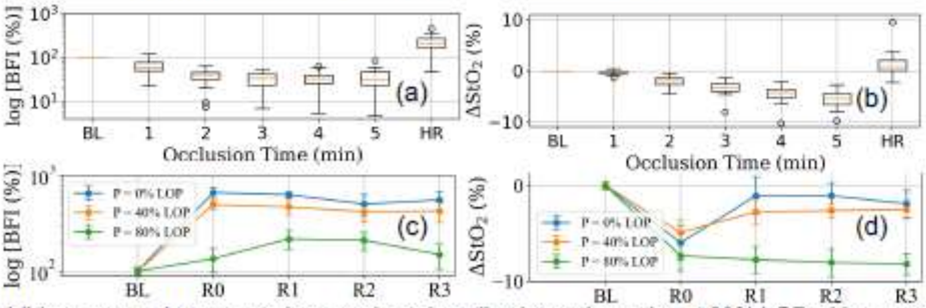

Fig 1: (a) and (b) correspond to our previous study on baseline hemodynamics at 80% LOP without exercise. Here, BL and HR correspond to baseline and hyperemic response respectively. (c) and (d) correspond to the hemodynamic study at 80% LOP with isometric ankle plantar flexion exercise. Here, BL and R correspond to baseline, rest periods respectively.

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Determination of the respiratory compensation point by detecting changes in intercostal muscles oxygenation by using near–infrared spectroscopy

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Introduction: This study aimed to evaluate if the changes in oxygen saturation levels at intercostal muscles (SmO_2–*m.intercostales*) assessed by near–infrared spectroscopy (NIRS) using a wearable device could determine the respiratory compensation point (RCP) during exercise.

Methods: Fifteen healthy competitive triathletes (eight males; 29±6 years; height 167.6±25.6 cm; weight 69.2±9.4 kg; VO_2–máx 58.4±8.1 $mL \cdot kg^{-1} \cdot min^{-1}$) were evaluated in a cycle ergometer during the maximal oxygen-uptake test (VO_2–máx), while lung ventilation (VE), power output (watts, W) and SmO_2–*m.intercostales* were measured. RCP was determined by visual method (RCP_{visual}: changes at ventilatory equivalents ($VE \cdot VCO_2^{-1}$, $VE \cdot VO_2^{-1}$) and end-tidal respiratory pressure ($PetO_2$, $PetCO_2$) and NIRS method (RCP_{NIRS}: breakpoint of fall in SmO_2–*m.intercostales*).

Results: During exercise, SmO_2–*m.intercostales* decreased continuously showing a higher decrease when VE increased abruptly (Figure 1). A good agreement between methods used to determine RCP was found (visual vs NIRS) at %VO_2-máx, VO_2, VE, and W (Bland–Altman test). Correlations were found to each parameters analyzed (r=0.854; r=0.865; r=0.981; and r=0,968; respectively. $p<0.001$ in all variables, Pearson test), with no differences ($p<0.001$ in all variables, Student's *t*-test) between methods used (RCP_{visual} and RCP_{NIRS}).

Discussion: We concluded that changes at SmO_2-*m.intercostales* measured by NIRS could adequately determine RCP in triathletes.

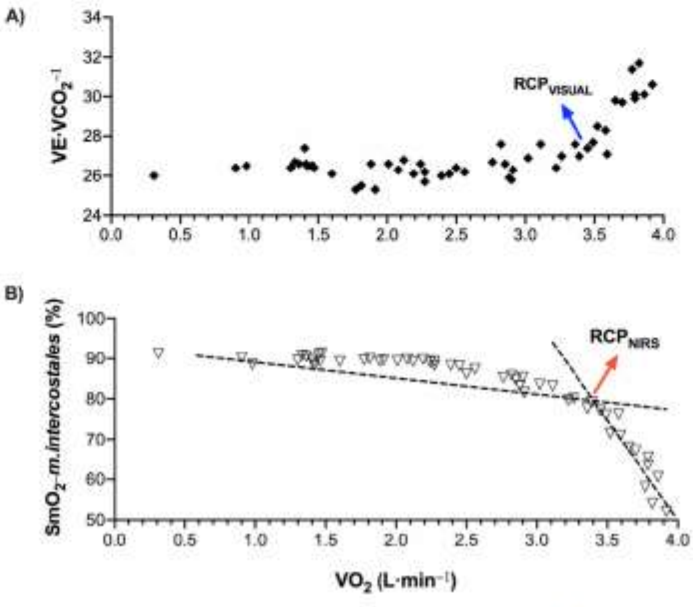

Figure 1. Example of RCP determination in a participant: (**A**) RCP_{visual}, (**B**) RCP_{NIRS}

Changes in oxygen muscle during high intensity exercise with or not support of high–flow nasal cannula

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Introduction: Exercise increases the cost of breathing (COB) due to increased lung ventilation (VE), inducing respiratory muscles deoxygenation (∇SmO_2), while the increase in workload implies ∇SmO_2 in locomotor muscles. This phenomenon has been proposed as a leading cause of exercise intolerance, especially in clinical contexts. The use of High–Flow Nasal Cannula (HFNC) during exercise routines in rehabilitation programs has gained significant interest because it is proposed as a therapeutic intervention for reducing symptoms associated with exercise intolerance, such as fatigue and dyspnoea, assuming that HFNC could reduce exercise–induced ∇SmO_2. We tested in a study with a cross–over design whether the muscular desaturation of *m.vastus lateralis* and *m.intercostales* during a high–intensity constant–load exercise can be reduced when it was supported with HFNC in non–physically active adults.

Methods: Eighteen participants (9 women; age: 22±2 years, weight: 65.1±11.2 kg, height: 173.0±5.8 cm, BMI: 21.6±2.8 $kg \cdot m^{-2}$) were evaluated in a cycle ergometer (15 min, 70% maximum watts achieved in ergospirometry (VO_2–peak)) breathing spontaneously (CONTROL, CTRL) or with HFNC support (HFNC; 50 $L \cdot min^{-1}$, fiO_2: 21%, 30°C), separated by seven days in randomized order. Two–way ANOVA tests analyzed the ∇SmO_2 (*m.intercostales* and *m.vastus lateralis)*, and changes in VE and $\nabla SmO_2 \cdot VE^{-1}$. Dyspnoea, leg fatigue, and effort level (RPE) were compared between trials by the Wilcoxon matched–paired signed rank test.

Results: We found that the interaction of factors (trial × exercise–time) was significant to ∇SmO_2–*m.intercostales*, VE, and (∇SmO_2–*m.intercostales*)/VE ($p<0.05$, all), but not in ∇SmO_2–*m.vastus lateralis*. ∇SmO_2–*m.intercostales* was more pronounced in CTRL during exercise since 5\` ($p<0.05$). Hyperventilation was higher in CTRL since 10\` ($p<0.05$). The $\nabla SmO_2 \cdot VE^{-1}$ decreased during exercise, being lowest in CTRL since 5\`. Lower dyspnoea was reported in HFNC, with no differences in leg fatigue and RPE.

Discussion: We concluded that wearable optical biosensors documented the beneficial effect of HFNC in COB due to lower respiratory ∇SmO_2 induced by exercise. We suggest incorporating NIRS devices in rehabilitation programs to monitor physiological changes that can support the clinical impact of the therapeutic intervention implemented.