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Preliminary *vastus lateralis* characterization with time domain near infrared spectroscopy during incremental cycle exercise

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ABSTRACT

Functional near infrared spectroscopy (NIRS) is a widespread non-invasive technique to monitor skeletal muscle metabolism. However, only variation of oxygenated (HHb), deoxygenated (O_2Hb), total (tHb) hemoglobin and saturation (SO_2) are usually reported. In this study, Time Domain (TD) NIRS approach was exploited to perform a preliminary quantitative characterization of *vastus lateralis* muscle during incremental exercise. A population of 11 healthy young male subjects performed on a mechanical cycle ergometer an incremental exercise (initial work rate range = 60-96 W, increment = 12-18 W/min) until exhaustion. TD NIRS, heart rate, pulmonary ventilation (VE), O_2 uptake (VO_2), CO_2 output (VCO_2), blood lactate concentration ([La]b) and Borg scale were measured during the exercise. From TD NIRS, muscles absolute values of absorption and scattering coefficients were obtained with a homogeneous approach and hemoglobin concentrations and saturation levels were calculated. The time courses of HHb, O_2Hb, tHb and SO_2 were consistent with previous literature results. A high inter-subject variability was found for both optical properties and hemodynamic concentrations. Further statistical group analysis will be required in order to highlight significant behavior within the population and correlation with physiological parameters.

Keywords: TD NIRS, muscle oxidative metabolism, vastus lateralis, optical properties, cycling exercise

1. INTRODUCTION

Functional near infrared spectroscopy (NIRS) is a diffuse technique to non-invasively investigate muscle oxidative metabolism at rest, during exercise or in response to different interventions (i.e. training, bed rest, hypoxia). Previous studies have investigated fractional O_2 extraction capacity and microvascular blood volume adaptations in both healthy subjects and patients with muscle impairments[1]. However, most of the studies reports relative variations of oxygenated and deoxygenated hemoglobin (O_2Hb and HHb) measured with the Continuous Wave (CW) NIRS approach, while there is a paucity of data on absolute concentrations changes. These information can be obtained by a Time Domain (TD) NIRS approach. It employs pulsed light at two different wavelengths in the range 600 – 1100 nm to retrieve absorption and scattering coefficients μa and μs' of the investigated tissues. Consequently, absolute values of O_2Hb, HHb, total hemoglobin (tHb) and saturation (SO_2) can be calculated[2]. In literature, few studies report optical parameters of skeletal muscles[3]. Here, we propose a preliminary study aimed to characterize optical proprieties of *vastus lateralis* muscle and evaluate functional adaptations to exercise of skeletal muscle oxidative metabolism in healthy subjects.

2. MATERIAL AND METHODS

2.1 Experimental protocol

Eleven untrained male subjects, age: 29 ± 6.7 years (mean ± SD) volunteered for the study and signed an informed consent. The experiments were conducted in accordance with the Declaration of Helsinki. Subjects performed a cycling incremental exercise to voluntary exhaustion with a mechanical flywheel cycle ergometer (Monark 818E; Stockholm, Sweden). After few minutes of rest, subjects started pedaling (60 revolutions per minutes) at an initial work rate of 60-96 W for 1 minute. The initial workload was selected according with the estimated level of physical fitness of each subject.

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Then, work rate was increased by 12-18 W every min until exhaustion, defined as the inability to maintain the pedaling frequency, despite encouragement by operators. At the end of the exercise, the recovery period lasted up to 15 min.

2.2 Physiological measurements

During the experiment, ECG was monitored and heart rate (HR) determined. Pulmonary ventilation (VE), O_2 uptake (VO_2) and CO_2 output (VCO_2) were assessed breath-by-breath using a computerized metabolic cart (SensorMedics Vmax29c; Bilthoven, The Netherlands). Blood lactate concentration ([La]b) was measured using an enzymatic method (Biosen C-line; EKF Diagnostic,) on 20 µl of capillary blood obtained at the ear lobe both at rest and at the end of exercise. Maximal level of self-perceived exertion was assessed using the validated Borg Scale. For more details about physiological measurements, see *Porcelli et al.*[4].

2.3 TD NIRS measurements and analysis

Tissue saturation, oxygenated and deoxygenated hemoglobin concentration changes in *vastus lateralis* muscle were evaluated at rest, during exercise and into the recovery. An in-house built TD NIRS device dedicated to muscle oxidative metabolism monitoring was used. As described by *Re at al.*[5], this device is equipped with two pulsed diode lasers (688 nm and 828 nm) and two TCSPC boards that process the signal coming by two hybrid photomultiplier tubes. Only one channel was employed for these measurements using a 3 cm distance between source and detection fibers and a sampling rate of 2 Hz (250 ms for each wavelength). Given the high diffusivity of biological tissues, the diffusion equation for a semi-infinite homogeneous medium, convolved with the instrument response function, was applied to fit the acquired reflectance curves. Characteristic absorption and scattering coefficients (μ_a and μ_s') were thus retrieved for every sampling point[6]. Subsequently, O_2Hb and HHb concentrations were calculated exploiting the Lambert-Beer's law and tHb and SO_2 values were derived.

3. RESULTS AND DISCUSSION

In Table 1, mean (± SD) absorption and scattering coefficients of *vastus lateralis* muscles of relevant intervals during the experiment (last 20 s of baseline, last 20 s of pedaling and last 20 s of recovery) are shown. Apparently, an increase of μ_a coefficients at 688 nm occurred at peak of incremental task with respect to the baseline, while at the end of the recovery period a return to the initial values was observed. On the other hand, a decreasing of μ_s' values seemed to happen during the task. These first observations needs to be assessed by further statistical analysis, evaluating both intra- and inter-subject variations. However, an inter-subjects high variability was observed (population % CV: μ_a > 25 % and μ_s' > 16%) suggesting potential influences of different muscle microstructure on these variables. In Table 1, absolute mean (± SD) values of HHb, O_2Hb, tHb and SO_2 in the same time intervals are reported for one representative subject. Time course of NIRS data, expressed as relative changes from resting condition, of the same subject are depicted in Figure 1. At the beginning of the exercise, an initial steep increase of HHb (blue curve) and a correspondent decrease of O_2Hb (red curve) was observed, while the total hemoglobin (green line) remained constant. Then, HHb and tHb progressively increased according to a higher metabolic request from skeletal muscle. After approximatively 9 min of incremental exercise, tHb and O_2Hb values decreased suggesting a cyclic impairment of microvascular perfusion due to the high muscle force production. HHb reached a maximal value (39.5 ± 1.74 µM) at exhaustion, as confirmed by other physiological parameters measured (HR = 93% predicted HRmax; VE = 93 l/min; VO_2 = 3.037 l/min; VCO_2 = 3.545 l/min ; [La]b = 9.11 mM; Borg scale = 17/20). SO_2 values progressively decreased during the exercise. In the recovery period, HHb returned to baseline values while tHb and O_2Hb remained elevated, possibly due to an increased perfusion following exercise.

4. CONCLUSION

Optical proprieties of *vastus lateralis* muscle and NIRS hemodynamics parameters related to skeletal muscle oxidative metabolism were investigated during incremental exercise in healthy subjects. Fractional O_2 extraction capacity and microvascular blood volume adaptations to exercise obtained by TD NIRS are in concordance with previous literature fundings[3]. Interesting additional information about quantitative values of optical properties have been retrieved. Further analysis will be required in order to highlight reproducible behavior among subjects and correlation with physiological variables.

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Table 1. Mean ± SD. values of μ_a and μ_s' of *vastus lateralis* muscle at 688 nm and 828 nm at different time points (average of 20 s of measurement).

	Baseline		*Peak exercise*		*Recovery*	
	688 nm	*828 nm*	*688 nm*	*828 nm*	*688 nm*	*828 nm*
$\mu_a\,[cm^{-1}]$	0.23 ± 0.06	0.26 ± 0.06	0.31 ± 0.12	0.25 ± 0.06	0.23 ± 0.07	0.26 ± 0.06
$\mu_s'\,[cm^{-1}]$	7.57 ± 1.21	6.23 ± 1.24	6.96 ± 1.39	6.09 ± 1.12	7.25 ± 1.18	6.00 ± 1.16

Table 2. Mean ± SD. values of hemoglobin concentrations and tissue saturation in a representative subject. 20 s intervals of baseline, peak exercise and recovery were considered.

	Baseline	*Task peak*	*Recovery*
HHb [μM]	26.8 ± 0.54	39.5 ± 1.74	28.4 ± 0.74
O_2Hb [μM]	80.0 ± 2.29	69.6 ± 2.28	83.1 ± 3.02
tHb [μM]	107 ± 2.02	109 ± 2.09	112 ± 2.55
SO_2 [%]	74.9 ± 0.82	63.8 ± 1.5	74.5 ± 1.11

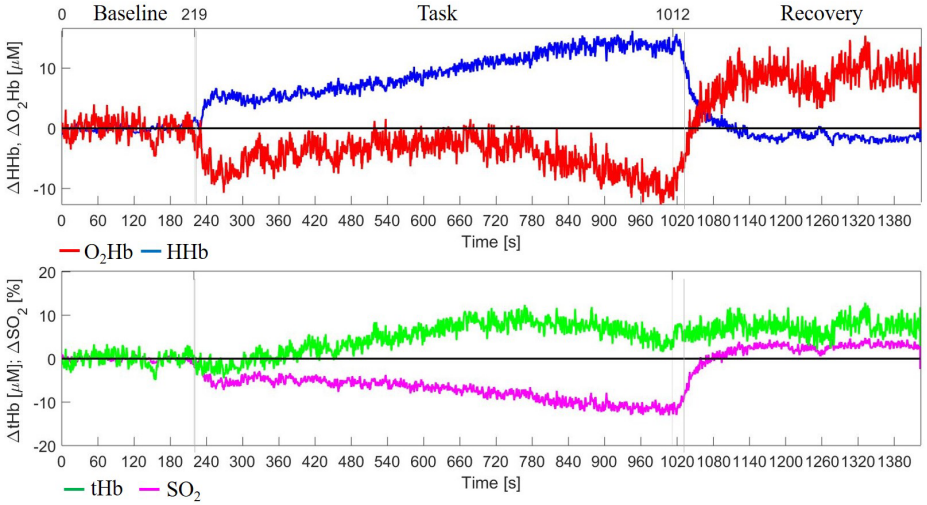

Figure 1. Representative subject's relative time course of TD NIRS hemodynamics parameters throughout the test.

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