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# Understanding Skeletal Muscle's Vascular Function and Health with Time-Domain Near Infrared Spectroscopy

Rebecca Re<sup>1,2,\*</sup>, Letizia Contini<sup>1</sup>, Caterina Amendola<sup>1</sup>, Davide Contini<sup>1</sup>, Lorenzo Spinelli<sup>2</sup> and Alessandro Torricelli<sup>1,2</sup>

<sup>1</sup>Dipartimento di Fisica, Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milan, Italy

<sup>2</sup>Istituto di Fotonica e Nanotecnologie, Consiglio Nazionale delle Ricerche, Piazza Leonardo da Vinci 32, 20133 Milan, Italy

\*rebecca.re@polimi.it

**Abstract:** We propose a method based on fast time domain near infrared spectroscopy to study skeletal muscle's vasculature function and health. We present the method and in-vivo acquisitions during rest, contraction and recovery on 8 subjects. © 2025 The Author(s)

## 1. Introduction

Near infrared spectroscopy (NIRS) application on musculoskeletal system mainly concerns the determination of the oxidative metabolism of working muscle, starting from the detection of the hemodynamic parameters related to the muscular tissue, i.e. oxy- (O<sub>2</sub>Hb) and deoxy- (HHb) haemoglobin, which helps understanding the metabolism requirement based on oxygen demand and extraction. In addition, it is possible to study hemodynamic parameters from another perspective: considering their oscillations, which occur for different physiological mechanisms. The study of hemodynamics oscillations comes from techniques such as laser doppler flowmetry (LDF) and photoplethysmography (PPG), which can probe just the more superficial part of the skin, with a penetration depth of few micrometers/millimeters, allowing for the assessment of skin microvasculature health. With continuous wave (CW) NIRS a higher penetration depth is guaranteed (up to 2-3 cm) and with time domain approach (TD) the depth selectivity is also improved, being able to retrieve the optical properties, and then the hemodynamic parameters, related to the muscular tissue, avoiding the confounding effects of the systemic vasculature [1]. By calculating the power spectral density (PSD) of the signals related to the haemodynamic parameters in the frequency range <2 Hz, it is possible to study the presence of characteristic frequency peaks associated with physiological and/or pathological phenomena. The spectral analysis of NIRS signal is not so usual in muscle-related applications. Around 2010, it was identified that CW NIRS spectrum can be divided into main bands related with different physiological origins [2], each of which gives information about a specific function of the vascular compartment: nitric-oxide (NO) independent- and dependent- endothelial activity (vasodilation), neurogenic activity due to vessel wall innervation (sympathetic nerve activity) and intrinsic myogenic activity of vascular smooth muscle (local control). In this work, we present, for the first time, the possibility to address this point with TD NIRS. This was possible thanks to recent technological advancements, which increased the SNR, allowing to perform measurements with up to 20 Hz acquisition rate at 4 cm of source-detector distance on phantoms with absorption coefficient of 1 cm<sup>-1</sup> [3]. To this purpose we perform fast acquisitions on anterior tibialis muscle of 8 healthy volunteers during rest, contraction and recovery. The measurement campaign is still running and foresees 20 subjects.

## 2. Material and methods

Fast TD NIRS acquisitions were performed employing a device previously developed at the Department of Physics, at Politecnico di Milano [3], with an acquisition rate of 20 Hz and 4 cm of source-detector distance. The anterior tibialis muscle was chosen because of the thick adipose tissue thickness (ATT), which allows data to be analyzed using the solution of the photon diffusion equation for homogeneous media. Subjects were set down with the ankle fixed onto a holder and connected to a load cell for measuring the traction force exerted. At first, they were asked to perform 3 consecutive dorsiflexion at the maximum force they were able to maintain, and the maximum voluntary contraction (MVC) was then calculated as their average. They performed a 400 s long dorsiflexion at the 40% of the MVC, which was preceded and followed by 400 seconds of rest and recovery respectively. Visual feedback of their force was shown to the participants on a screen, to help them in maintaining the force constant. The protocol was chosen based on numerical simulations previously performed [4]. The time course of the exerted force from the dynamometer was obtained to have feedback on the exercise execution during the post processing of data. Absolute values for the time courses of oxy- (O<sub>2</sub>Hb) and deoxy- (HHb) hemoglobin concentrations were calculated from the absorption coefficients through 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) was calculated for all the physiological and hemodynamics parameters during rest, contraction and recovery phase. The mean PSD power in frequency bands VI-I (see Table 1) was derived and divided by the total PSD, calculated over the whole spectrum (0-10 Hz) and by the band amplitude.

Table 1. Association of frequency spectral intervals with physiological origin in the range 0.005-2 Hz [5].

Physiological Origin	Frequency Interval	
	[Hz]	Symbol
Endothelial	0.005-0.0095	VI
Nitric oxide (NO)-related endothelial	0.0095- 0.021	V
Neurogenic	0.021-0.052	IV
Myogenic	0.052-0.145	III
Respiratory	0.15- 0.6	II
Cardiac	0.6-2	I

### 3. Results and discussions

The mean age of the healthy subjects was  $27.4 \pm 1.3$  years; the mean ATT was  $5.88 \pm 1.43$  mm. Observing the time course of the force signal, we can describe two different typical behaviors among the subjects acquired: subjects who were able to maintain the force for the whole contraction, as shown in Fig. 1a (black line almost constant) and subjects who were not able to maintain the force at the expected value for the full contraction length, as shown in Fig. 1b, black line. In these figures we also present the corresponding time courses for the O2Hb (red) and HHb (blue) relative to the anterior tibialis. For visualizing purposes, the haemodynamic time courses are shown with the baseline subtracted, calculated over the last 10 seconds during rest; while the force is normalized to its maximum value, secondary axis.

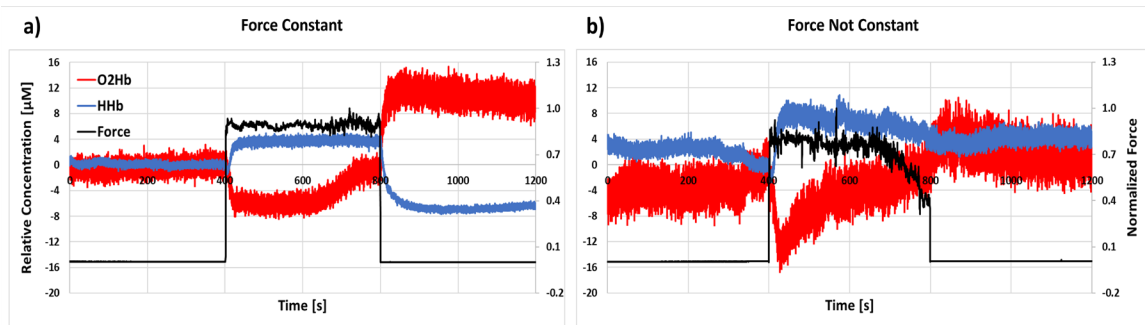


Fig. 1. Time courses of the O2Hb and HHb of two typical subjects: a) the force (black) was maintained constant during the dorsiflexion and b) the force was not maintained constant.

In Fig. 2, the average PSD in the six spectral regions of interest, for the two subjects proposed in Fig.1, are presented for O2Hb and HHb. These spectra are well representative of the two identified groups. For all the subjects, during rest, most of the 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. In addition, the PSD for BVP is similar to O2Hb and HHb, while a strong contribution to band II is found in the PSD for RESP, as expected. During the contraction we can notice common behavior for those subjects who didn't maintain the force constant: a huge increase in the VI and III bands for both O2Hb and HHb and a corresponding decrease during the recovery phase. This behavior could suggest that during contraction a mechanism of vasodilation, not mediated by NO, and local control (myogenic activity) of this phenomenon occurs. In the subjects who maintained the force constant, we observe changes in band VI during the contraction, but the sign of these changes is not always the same, while smaller variations in band III are present. In both cases there are changes related to the contraction in band V and IV, but of smaller amplitude, while no big changes are observed in band II and I, as expected. Of course, the number of subjects is still too limited for deriving general behavior conclusions inside the different bands and performing statistics, but we are increasing the number of subjects involved.

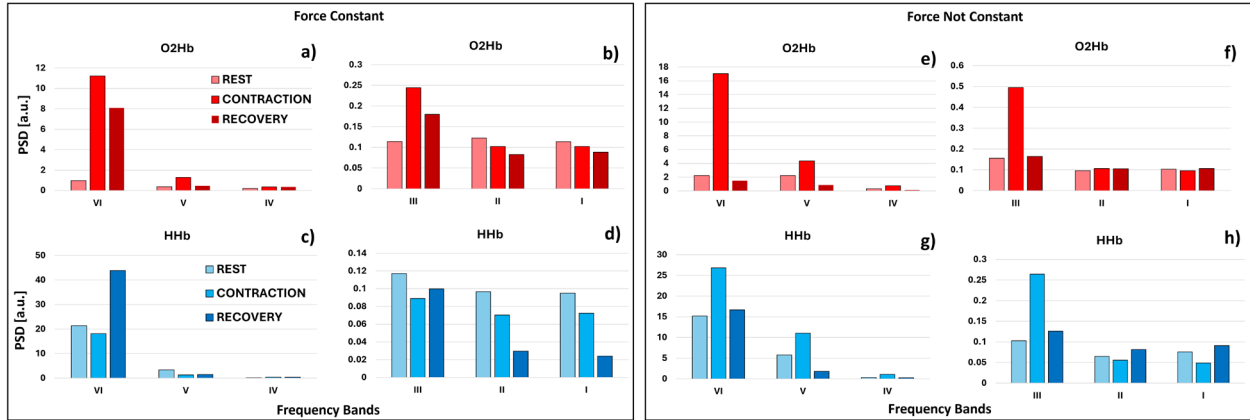


Fig. 2. Power spectral density (PSD) in the six frequency ranges of interest, in arbitrary unit, for O2Hb (red) and HHb (blue) in different phases: rest, contraction (dorsiflexion) and recovery, measured on the anterior tibialis muscle of the two typical subjects. a-d: subject able to maintain the contraction for the whole session, e-h: subject not able to maintain the contraction.

### 3. Conclusion

In conclusion, after overcoming technological difficulties in the increasing of the SNR in TD NIRS measurements [3] and performing numerical simulations for understanding the best protocol for fast TD NIRS acquisitions [4], we performed the first fast in-vivo acquisitions on the anterior tibialis muscle, confirming the possibility of this kind of measurement. We propose a new way to analyze muscular TD NIRS data, based on the interpretation of their PSD, to have information about the muscle's vasculature health and the mechanism that are driving muscular contraction. This method is based on the consideration of different frequency bands which are related to different physiological origins. Further investigations and the increasing number of participants, already in progress, will allow to trace general considerations and will strongly impact in future muscular physiological studies: with one unique acquisition it will be possible to add these new information to the more traditional oxidative metabolism NIRS outcome.

### 3. References

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