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# Differences of Time-Domain NIRS-derived Hemodynamic Parameters of the Vastus Lateralis Muscle with Age and Physical Activity

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**Abstract:** Skeletal muscle hemodynamic parameters of 59 volunteers from two age groups (middle-aged and old) and two training levels were measured using Time-Domain Near-Infrared Spectroscopy. Significant differences were observed with training status, but not with age. © 2025 The Author(s)

## 1. Introduction

Aging leads to a progressive loss of skeletal muscle mass and function, which represents a major concern for the health and independence of aging individuals [1]. Such neuromuscular decline accelerates after the age of 50 and leads to higher hospitalization rates [2]. The "Trajector-AGE" project focuses on the study of neuromuscular decline in aging with a multimodal approach to give a comprehensive characterization of the muscular status and to trace trajectories of aging on a cohort of 100 subjects in two age ranges: middle-aged (55-60 years old) and old (70-80 years old) [3].

In this preliminary work, we investigate the ability of Time-Domain (TD) Near-Infrared Spectroscopy (NIRS) to detect differences, if any, in the hemodynamic parameters of the *vastus lateralis* muscle between middle-aged and old populations, as well as between sedentary and trained old subjects, in a subgroup of 59 subjects. A vascular occlusion test (VOT) was performed to investigate muscle function.

## 2. Materials and methods

### *2.1. Measurement protocol*

Fifty-nine physically and cognitively healthy male volunteers were divided into two age groups: middle-aged (19 subjects, 59.0 $\pm$ 4.0 years old) and old (40 subjects, 74.7 $\pm$ 5.7 years old, 23 sedentary and 17 trained).

Measurements on sedentary volunteers were carried out using a TD NIRS device previously developed at Politecnico di Milano (system 1) [4], while for the trained subjects a commercial TD NIRS system (PIONIRS s.r.l., Milan, Italy) was employed (system 2). In both cases the optical fibers, placed at a source-detector separation of 2.5 cm, were hosted in flexible custom-made optical probes, which were positioned on the distal *vastus lateralis* muscle and secured using black auto-adhesive bandages. The acquisition rate of system 1 was 0.8 Hz, while that of system 2 was 10 Hz.

During the measurements, the subjects were seated, with their right leg still in a fixed position. After an initial baseline (at least 120 s), a pneumatic cuff placed at the inguinal crease of the thigh was inflated at a pressure of 280-300 mmHg to occlude the femoral artery. The cuff was deflated when the level of oxygen saturation reached a plateau (with a maximum occlusion time of 300 s). The recovery was monitored for at least 180 s.

In addition to the optical data, the adipose tissue thickness (ATT) above the *vastus lateralis* was also measured, using a skinfold caliper in the case of sedentary subjects and ultrasound for the trained ones.

Six month after the initial evaluation (T0), 48 out of the 59 volunteers repeated the same assessment at a second time point (T1).

### 2.2. *Data analysis*

The TD NIRS data was analyzed using the solution of the diffusion equation for homogeneous semi-infinite media, and Beer's Law was used to calculate the absolute concentrations of oxygenated hemoglobin ($HbO_2$, expressed in µM), deoxygenated hemoglobin (HHb, expressed in µM) and tissue oxygen saturation ($StO_2$, expressed in %). A 5-s moving average filter was applied to all signals, and the following parameters were then extracted:

- **Baseline**: the baseline of each signal was calculated as the average over the last 10 seconds before the start of the occlusion.
- **Slope 1**: the slope of the signals during the first 60 seconds of the occlusion, calculated via a linear fit.
- **Slope 2**: the slope of the signals during the first 10 seconds of the recovery (re-perfusion), calculated via a linear fit.

The following comparisons were carried out:

- **Cross-sectional analysis of sedentary subjects**: within the sedentary group, the parameters measured at T0 in the middle-aged and old subjects were compared. The Mann-Whitney U test was used to assess possible differences.
- **Cross-sectional analysis of old subjects**: the parameters measured at T0 in the sedentary old subjects were compared to those measured in the trained ones. The Mann-Whitney U test was used to assess possible differences.
- **Longitudinal analysis**: for the subjects from all groups who attended both measurement sessions, the parameters measured at T0 and T1 were compared, using the Wilcoxon signed-rank test to assess significance.

In all cases, the threshold for significance was set to $p < 0.05$.

## 3. Results

Out of the 59 subjects, 48 exhibited expected signal trends (e.g., during the occlusion period HHb increased, while $HbO_2$ and $StO_2$ decreased) while 11 showed unexpected behaviors (e.g., $HbO_2$ increasing during the occlusion period), as shown in Figure 1. This could be due to the ATT, which was higher in the latter group (6.5 mm ± 4.4 mm) than the former (3.6 mm ± 2.3 mm), impeding a complete arterial occlusion. The subjects with anomalous signals were excluded from further analysis, resulting in the following sample: 12 middle-aged subjects (age 58.4 years ± 3.7 years, ATT 3.7 mm ± 1.0 mm), 21 sedentary old subjects (age 78.0 years ± 4.7 years, ATT 4.0 mm ± 3.4 mm), and 15 trained old subjects (age 70.1 years ± 4.4 years, ATT 3.1 mm ± 1.7 mm).

The cross-sectional analysis of the sedentary subjects yielded no significant differences for any of the parameters between middle-aged and old subjects.

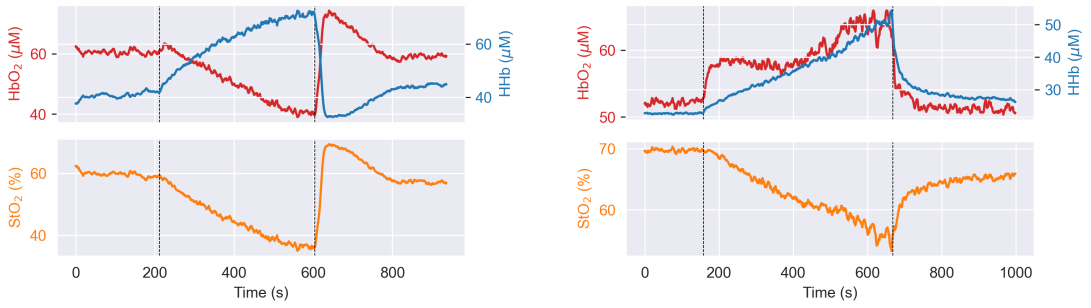


Fig. 1: Examples of measured absolute values of $HbO_2$ (red), HHb (blue) and $StO_2$ (orange) for the vastus lateralis of a middle-aged subject during a good VOT (panel a) and a sedentary old subject during a bad VOT (panel b). The dashed vertical lines indicate the start and end of the occlusion period.

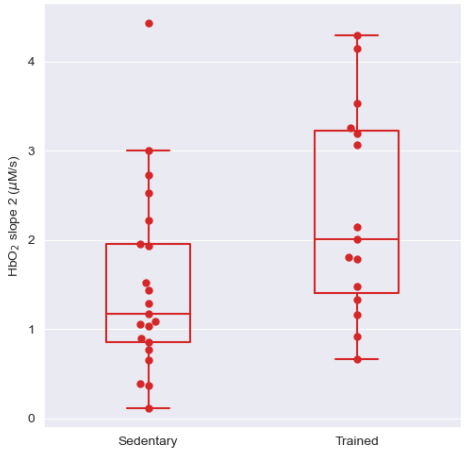


Fig. 2: Boxplots showing the difference ($p = 2.9 \times 10^{-2}$) in HbO slope 2 between sedentary (left) vs. trained (right) old subjects.

For the cross-sectional analysis of old subjects, the trained group exhibited higher baseline $HbO_2$ ($p = 8.5 \times 10^{-4}$), higher $HbO_2$ slope 2 ($p = 2.9 \times 10^{-2}$) (Figure 2), higher baseline $StO_2$ ($p = 7.0 \times 10^{-3}$), and lower absolute value of $StO_2$ slope 1 ($p = 4.3 \times 10^{-2}$).

In the longitudinal analysis, no significant time-related differences were observed for either age group and training status.

## 4. Discussion and conclusions

The absence of significant differences between sedentary middle-aged and old subjects seems to suggest no age-related impairment in vascular function. However, the lack of observed differences could arise from the exclusion criterion: indeed, by discarding the bad occlusions, the sample was biased in favor of the subjects with lower ATT, reducing inter-group variability.

Conversely, significant differences were observed between sedentary and trained old subjects. In particular, the higher re-perfusion slope for $HbO_2$ indicates better vascular function in trained individuals compared to their sedentary counterparts.

The lack of significant differences in the longitudinal analysis suggests that no changes in muscle function occurred over a 6-month period.

## 5. Acknowledgments

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