Spatial distribution of vastus lateralis blood flow and oxyhemoglobin saturation measured at the end of isometric quadriceps contraction by multichannel near-infrared spectroscopy

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1 Introduction

Near-infrared spectroscopy (NIRS) has found a number of applications in the noninvasive study of hemodynamic- and oxygenation-related parameters in tissue. For example, the focal modifications in the cerebral hemodynamics induced by specific stimulation paradigms1 and the effects of exercise on muscle oxygenation2,3 have both been investigated with NIRS. Furthermore, NIRS affords the measurement of regional muscle blood flow (MBF) and oxygen consumption ($\text{VO}_2$) according to the venous or arterial occlusion methods.4,5 Multichannel NIRS instruments allow for spatial imaging or multisite measurements, which are of crucial importance in applications aiming at the detection of localized modifications to the blood flow and VO$_2$ (for instance, ischemic, hyperperfused, or hypoperfused tissue regions). So far, a number of multichannel NIRS applications have been devoted to the study of evoked brain activation, which typically induces an increase and a decrease in the regional cerebral concentrations of oxy-hemoglobin ($\text{O}_2\text{Hb}$) and deoxy-hemoglobin (HHb), respectively.1 Multisite measurements are also relevant in the investigation of the MBF and VO$_2$ spatial variability. In particular, the feasibility of multichannel NIRS measurements to investigate the spatial variability of the regional MBF and/or VO$_2$ at rest in skeletal muscle has been previously reported using multichannel, frequency-domain, near-infrared photometers, capable of measuring the optical pathlength in tissue.6–9 Although the spatial variability of muscle oxygenation has been recently reported also using different prototypes of multichannel, continuous-wave, near-infrared photometers,10–13 only frequency-domain or time-domain near-infrared photometers can provide an absolute measurement of muscle oxygenation as well as MBF/VO$_2$.

Single-point measurements of MBF, even during exercise, are possible using the light-absorbing tracer indocyanine green.14–16 The heterogeneity of MBF during dynamic and isometric exercise has been recently measured in the quadriceps by a positron emission tomography imaging study.17 However, the invasiveness of these two methods limits their applicability.

In this study, we measured noninvasively the spatial distribution of vastus lateralis MBF under rest condition and immediately postexercise, and we monitored the total hemoglobin concentration ($t\text{Hb} = \text{O}_2\text{Hb} + \text{HHb}$) and muscle oxygen saturation over a 4 $\times$ 8 cm$^2$ area of the vastus lateralis at rest and immediately after isometric, maximal quadriceps contraction using multichannel, frequency-domain, near-infrared spectroscopy. A venous occlusion was applied 20 s before the end of the exercise, so that the venous-occlusion-induced increase in total hemoglobin was recorded without any delay after the end of the exercise. Therefore, we were able to investigate the relationship between the exercise-induced changes in vastus lateralis MBF and SmO$_2$. After exercise, MBF increased significantly at each measured location. Comparing the MBF values measured at the end of exercise in the proximal and distal regions, we observed that only one proximal region had a significantly higher MBF than the corresponding distal one. The maximum desaturation measured during exercise was positively correlated with the postexercise to pre-exercise MBF ratio in both the proximal ($P = 0.016$) and distal ($P = 0.0065$) regions. These data confirm that frequency-domain tissue oximeters are noninvasive, powerful tools to investigate the spatial and temporal features of muscle blood flow and oxygenation, with potential applications in areas of pathophysiology. © 2004 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.1646417]

Keywords: near-infrared; muscle; exercise; frequency-domain; muscle oxygen saturation.

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Abstract. Muscle blood flow (MBF) and muscle oxygen saturation (SmO$_2$) were measured at eight locations (four proximal, four distal) over a 4 $\times$ 8 cm$^2$ area of the vastus lateralis at rest and immediately after isometric, maximal quadriceps contraction using multichannel, frequency-domain, near-infrared spectroscopy. A venous occlusion was applied 20 s before the end of the exercise, so that the venous-occlusion-induced increase in total hemoglobin was recorded without any delay after the end of the exercise. Therefore, we were able to investigate the relationship between the exercise-induced changes in vastus lateralis MBF and SmO$_2$. After exercise, MBF increased significantly at each measured location. Comparing the MBF values measured at the end of exercise in the proximal and distal regions, we observed that only one proximal region had a significantly higher MBF than the corresponding distal one. The maximum desaturation measured during exercise was positively correlated with the postexercise to pre-exercise MBF ratio in both the proximal ($P = 0.016$) and distal ($P = 0.0065$) regions. These data confirm that frequency-domain tissue oximeters are noninvasive, powerful tools to investigate the spatial and temporal features of muscle blood flow and oxygenation, with potential applications in areas of pathophysiology. © 2004 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.1646417]
saturation ($\text{SmO}_2$) during isometric, maximal contractions. For this purpose, we used a multichannel NIRS instrument, allowing for simultaneous measurements at eight locations. Because the MBF response to exercise is characterized by an increase followed by a rapid decrease toward the pre-exercise value when the exercise is stopped, it is important to perform a MBF measurement immediately following the end of the exercise. For this reason, we applied the venous occlusion 20 s before the end of the exercise, so that we were able to record the venous-occlusion-induced increase in tHb without any delay after the end of the exercise. Therefore, the relationship between the exercise-induced changes in vastus lateralis $\text{SmO}_2$ and in MBF was correctly investigated.

2 Methods

The experiments were carried out on five subjects (age: 24.6 ± 6.4 y). The study was approved by the Institutional Review Board of Tufts University, where the experiments were performed, and all subjects gave their written informed consent. NIRS measurements were performed on the right vastus lateralis muscle using a multichannel, two-wavelength (690 and 830 nm) frequency-domain tissue oximeter (OxiplexTS, ISS, Champaign, IL). The design and the general features of this device have been described elsewhere. Briefly, the modulation frequency of the light-source intensity is 110 MHz, and the cross-correlation frequency for heterodyne detection is 5 kHz. The light sources and the optical detectors are all coupled to optical fibers; multimode glass fibers (400 μm in core diameter) for the light sources, and fiber bundles (3 mm in internal diameter) for the optical detectors. The configuration used for this experiment features 32 light sources (laser diodes: 16 emitting at 690 nm, 16 at 830 nm) and four independent detector channels. The four parallel detection channels consist of four photomultiplier tube detectors whose current outputs are converted to voltage, bandpass filtered, amplified, and directed to a four-channel, 16-bit analog/digital acquisition card. These four independent detection channels are time shared by the 32 laser diodes, which are multiplexed at a frequency of 100 Hz. In other words, the laser diodes are turned on and off in sequence with an on-time per diode of 10 ms. As a result, the time required to cycle through the whole set of 32 laser diodes is 320 ms. In the experiments reported here, we have averaged the data collected over four 320 ms cycles, thus obtaining an overall acquisition time per data point of 1.28 s. The optical probe arranges the optical fibers for light delivery to and collection from the tissue as illustrated in Fig. 1. There are two pairs of illumination optical fibers to the left (for measurements at proximal regions $P_1$, $P_2$, $P_3$, $P_4$) and to the right (for measurements at distal regions $D_1$, $D_2$, $D_3$, $D_4$) of each collection optical fiber (large filled circles in Fig. 1). These eight measured regions (four proximal and four distal) are distributed over an area of about 4 cm × 8 cm. Each pair of illumination optical fibers delivers light at 690 nm (small filled circles in Fig. 1) and 830 nm (small open circles in Fig. 1). The two pairs of illumination optical fibers are placed at distances of 2.5 and 4.0 cm, respectively, from the corresponding collection fiber (multidistance scheme). The four collection optical fibers are separated by 1.0 cm from each other.

![Illustration of the optical probe placement over an area (4 × 8 cm²) of the right vastus lateralis muscle. Schematic diagram of the optical probe showing the geometrical arrangement of the source and the detector optical fibers.](image)

The geometrical arrangement of the optical fibers allows for the implementation of two schemes of data analysis. (a) The absolute measurement of the tissue absorption and reduced scattering coefficients using the frequency-domain multidistance method at each proximal ($P_1$, $P_2$, $P_3$, $P_4$) and distal ($D_1$, $D_2$, $D_3$, $D_4$) measured location. (b) The relative measurement of changes in the tissue concentrations of $\text{O}_2\text{Hb}$ and $\text{HHb}$ (expressed in micromolar) using data from a given source-detector pair and a modified Beer–Lambert approach based on the knowledge of a differential pathlength factor (DPF). The multidistance scheme for absolute measurements has been tested in vitro on tissue-like phantoms, and in vivo on both animal models, and human subjects. In particular, absolute measurements of tissue oxygen saturation showed an excellent correlation with the data from co-oximetry in the in vivo studies. In this study, we have combined the frequency-domain, multidistance (2.5 and 4.0 cm) measurements (to determine the differential pathlength factor) and single-distance (4.0 cm) intensity measurements (to apply the modified Beer–Lambert law), as previously described. Specifically, we initially determined the absorption and scattering coefficients at each wavelength and at each proximal and distal measured location during baseline using multidistance data; then, we used these base line optical coefficients to calculate the DPF at each wavelength/location, and the temporal recordings of the optical intensity at a single source-detector separation of 4 cm to measure the changes in the concentrations of $\text{O}_2\text{Hb}$ and $\text{HHb}$ at each location during the exercise/venous-occlusion protocol. The local measurement of the DPF at both wavelengths (as opposed to assuming a DPF value from literature data) is important to obtain accurate recordings of the relative changes of $\text{O}_2\text{Hb}$ and $\text{HHb}$ concentrations. Furthermore, an absolute base line measurement of tissue hemoglobin saturation is required to quantify the changes in muscle saturation during the protocol. We opted for single-distance, relative measurements during the...
exercise/venous-occlusion protocol because of the high signal-to-noise ratio of intensity measurements, and because of the potentially better performance of single-distance (versus multidistance) measurements for mapping spatial inhomogeneities. The source-detector distance of 4.0 cm was chosen because it probes deeper tissue, thus including a larger fraction of muscle tissue, than the 2.5 cm distance.

NIRS measurements were performed on volunteers in a comfortable supine position. The optical probe was attached to the skin overlying the lower one-third of the vastus lateralis, with the line containing the four detection locations perpendicular to the major axis of the thigh (see Fig. 1). The optical probe, which covered an area of about 4 x 8 cm², was secured by wide elastic bands wrapped around the thigh of the subject (not too tight to avoid blockage of blood flow). No downward sliding of the probe was observed at the end of the measurements in any subject. Adipose tissue thickness underlying the monitored areas of the muscle groups was measured with a skinfold caliper. Adipose tissue thickness was 4.5 ± 0.8 mm and 5.1 ± 1.3 mm in the proximal and distal areas of the vastus lateralis, respectively. Considering that the thickness of the adipose tissue at the investigated area was less than 6 mm, it can be assumed that the measured variations in SmO₂ reflect metabolic changes occurring mainly in the muscle tissue.²⁷ In addition, the similar thickness of adipose tissue at the proximal and distal locations allows for a meaningful comparison of the SmO₂ values at the eight measured locations.

The measured value of the vastus lateralis SmO₂ reflects predominantly the weighted mean of arteriolar, capillary, and venular oxygen saturations with a minor (less than 20%) contribution from myoglobin (Mb).²⁷ Right vastus lateralis MBF was measured from the rate of change in tHb upon venous occlusion (Fig. 2) obtained by inflating to a pressure of 65 mm Hg a tourniquet placed around the upper thigh. The right thigh was at the heart level and the lower leg at an upward angle of 10°-15° in order to allow for a rapid venous drain-

gage after each venous occlusion. The foot was supported so that there was no contact between the leg and the bed, and as a result, the circulation in the leg was completely unrestricted. The venous occlusion was maintained for a time of about 60 s and was performed four times at about 3 min intervals before the isometric exercise. MBF was calculated according to the method previously described by De Blasi et al. and van Beekvelt et al.²⁸,²⁹ from the initial rate of increase in tHb during venous occlusion. Because this initial rate of tHb raise is eventually slowed down by the pressure buildup in the muscle, we considered the maximum rate of increase of tHb (measured as the slope of a linear fit of tHb data over a time of 12.8 s, or 10 data points) during the first 25 s of venous occlusion. We found this automated approach to the calculation of the blood flow from the temporal trace of tHb during venous occlusion to be robust and reliable. This approach is expressed by the following equation:

\[
\text{MBF} = \frac{1}{C} \frac{d(tHb)}{dt}_{\text{max}},
\]

where \(C\) is the hemoglobin concentration in the blood (for which we assume a value of 2.3 mM), and "max" refers to the maximum value over the first 25 s of venous occlusion. The MBF data in this study are expressed in mL (blood)/100 mL (tissue)/min. We also calculated the ratio between the MBF measured immediately after exercise and the mean MBF value measured before exercise. In each subject, the MBF mean value before exercise is the average of four measurements, corresponding to four successive venous occlusions.

The subject contracted his quadriceps isometrically and maximally for 30 s. Subject was verbally requested to obtain the maximal performance. Three repetitions of the exercise were performed, separated by 180 s of rest. About 20 s before the end of each exercise, the thigh tourniquet was inflated to a pressure of 65 mm Hg. During muscle contraction, the local pressure increase induces a regional vascular occlusion that accounts for the fact that the cuff inflation has a minimal effect during exercise. After the end of the exercise, the cuff was maintained inflated for about 30 s to induce a venous occlusion that allows for the measurement of MBF immediately postexercise. We report only the results of the contraction (1 out of 3) associated with the highest desaturation level. Maximal desaturation in the exercising muscle was calculated by taking the difference between the absolute values of SmO₂ measured at rest (mean value over the 2 min base line) and at the end of exercise (mean value over the last 5 s of exercise).

Mean and standard deviation of SmO₂ and MBF values within the proximal and distal regions were determined separately and compared using repeated measures analysis of variance. Significant differences were identified using Tukey’s honestly significant difference multiple comparison test. The paired t-test was used to compare the SmO₂ and MBF values corresponding to each pair of proximal and distal positions. The correlation between changes in SmO₂ and the MBF ratio was expressed by the Pearson’s correlation coefficient. Data are presented as mean ± SD. The criterion for significance was \(P < 0.05\).
Results

Figure 2 shows the typical temporal trace of tHb kinetics induced by repeated venous occlusion maneuvers at each measurement region. We found these changes to be reproducible at each of the eight measurement regions. The grand average of the MBF values measured at rest for each measured region of the vastus lateralis is reported in Table 1. Under rest conditions, MBF showed little variability between the proximal and the distal regions, with marginally lower values in the proximal regions. Specifically, comparing rest MBF values in the proximal \((P)\) and the distal \((D)\) regions, we observed that only \(P2\) and \(P3\) are significantly lower than \(D2\) \((P = 0.0485)\) and \(D3\) \((P = 0.0128)\), respectively.

The time course of SmO2 and tHb corresponding to the region \(P1\) of the right vastus lateralis muscle during isometric, maximal leg contraction (data from subject No. 2) is shown in Fig. 3. SmO2 at rest was about 68% for subject No. 2. At rest, considering all subjects, SmO2 was uniform over the distal region and over the proximal region, except for position \(P3\), where SmO2 was significantly higher than in region \(P1\) \((P < 0.05)\). Comparing SmO2 values in the proximal and the distal regions, only \(P2\) was significantly lower than \(D2\) \((P = 0.0057)\). The lack of significant difference in the other measurement points could be due to the intersubjects

### Table 1

<table>
<thead>
<tr>
<th>Proximal region</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
</tr>
</thead>
<tbody>
<tr>
<td>At rest</td>
<td>0.5±0.4</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>After end-exercise</td>
<td>2.0±1.3a</td>
<td>2.1±0.6a</td>
<td>1.7±0.2a</td>
<td>1.6±0.4a</td>
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<tr>
<td>At rest</td>
<td>0.6±0.3</td>
<td>0.6±0.3b</td>
<td>0.6±0.2c</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>After end-exercise</td>
<td>1.8±1.5</td>
<td>1.5±0.5a,d</td>
<td>1.4±0.5a</td>
<td>1.5±0.7a</td>
</tr>
</tbody>
</table>

### Distal region

<table>
<thead>
<tr>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>At rest</td>
<td>63.0±5.5</td>
<td>64.6±6.3</td>
<td>71.6±10.1b</td>
</tr>
<tr>
<td>End-exercise</td>
<td>48.8±8.9a</td>
<td>43.6±6.4a</td>
<td>56.4±14.0c,d</td>
</tr>
<tr>
<td>At rest</td>
<td>63.6±9.1</td>
<td>68.3±6.7c</td>
<td>66.5±4.2</td>
</tr>
<tr>
<td>End-exercise</td>
<td>58.4±6.3</td>
<td>58.3±5.2c,d</td>
<td>56.1±5.0c</td>
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### Table 2

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<th>P3</th>
<th>P4</th>
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<tbody>
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<td>69.0±6.8</td>
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</tr>
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</table>

\(a\) Significantly different from the corresponding value at rest.

\(b\) Significantly different from \(P1\).

\(c\) Significantly different from \(P2\) at rest.

\(d\) Significantly different from \(P2\) after end of exercise.
variability of the SmO₂ and/or the low number of the subjects.

The initial tHb response to the quadriceps exercise was quicker than the SmO₂ response (Fig. 3) as a result of the sudden increase of the intramuscular fluid pressure that removes part of the blood from the vessels of the exercising muscle.¹⁰ The value of tHb started dropping at the onset of the contraction, and reached a stable value until the end of the exercise. A small increase in tHb was observed when the thigh tourniquet was inflated. By contrast, SmO₂ gradually decreased reaching its minimum value at the end of the muscle contraction. As expected, at the end of the exercise, tHb promptly increased overshooting the pre-exercise value. SmO₂ gradually recovered and returned to the pre-exercise value within about 1 min.

At the end of the maximal isometric contraction, SmO₂ decreased significantly in all the measurement points of the proximal region and in two (namely, D₂ and D₃) of the four measurement points of the distal region (Table 2). At the end of the isometric exercise, SmO₂ was uniform over the distal region and over the proximal region, except for position P₃ and P₄ where SmO₂ was significantly higher than in position P₂ (P<0.05). Comparing the SmO₂ values measured at the end of the exercise in the proximal and the distal regions, only P₂ was significantly lower than D₂ (P=0.0277).

Immediately after the end of the isometric exercise, MBF was uniform over the proximal and the distal regions (Table 1). However, MBF increased significantly, with respect to the rest value, at each location in the proximal region and in three (namely, D₂, D₃, and D₄) of the four distal regions. Comparing the MBF values measured at the end of the exercise in the proximal and the distal regions, we found that only P₂ was significantly higher than D₂ (P=0.0032).

The MBF postexercise to pre-exercise ratio was uniform within the proximal and the distal regions (Table 3). Comparing the MBF ratio in the proximal and distal regions, we found that only P₂ was significantly higher than D₂ (P=0.0291), and P₃ was significantly higher than D₃ (P=0.0255).

The maximum desaturation (ΔSmO₂) measured during exercise was positively correlated with the postexercise to pre-exercise MBF ratio in the proximal (P=0.016) and distal (P=0.0065) regions (Fig. 4).

### 4 Discussion

Using a commercial multichannel, frequency-domain, near-infrared photometer capable of measuring the optical pathlength in tissue, several interesting findings were observed in the present study. First, for the first time, it was reported that it is possible to map MBF simultaneously over a 4×8 cm² surface of the human quadriceps immediately after isometric maximal contraction; second, as expected, MBF after exercise was found significantly increased with respect to baseline; third, the postexercise to pre-exercise MBF ratio was uniform within the proximal and the distal regions. However, the MBF ratios at regions P₂ and P₃ were significantly higher than those at D₂ and D₃, respectively; fourth, the maximum desaturation measured during exercise was positively correlated with the postexercise to pre-exercise MBF ratio in the proximal and distal regions.

So far, most of the NIRS muscle studies have been performed on single locations demonstrating the usefulness of this noninvasive technique to evaluate muscle oxidative metabolism and/or hemodynamics at rest and/or during static or dynamic exercise in healthy subjects or patients.²³ However, the monitoring of single muscle locations is not representative.

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**Table 3** Right vastus lateralis MBF ratio values over the eight measurement points (n=5). (Data are presented as mean ±SD.)

<table>
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<th>P3</th>
<th>P4</th>
</tr>
</thead>
<tbody>
<tr>
<td>After end-exercise/at rest</td>
<td>5.0±0.8</td>
<td>7.2±2.8</td>
<td>6.0±2.3</td>
<td>5.5±2.4</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Distal region</th>
</tr>
</thead>
<tbody>
<tr>
<td>D₁</td>
</tr>
<tr>
<td>3.0±1.5</td>
</tr>
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</table>

<sup>a</sup> Significantly different from P₂.<br><sup>b</sup> Significantly different from P₃.
of the heterogeneous oxidative metabolic responses to the
same exercise either within the same muscle group or among
different muscle groups. The ongoing development of multi-
channel NIRS devices (imagers) offers the great advantage to
measure concomitantly different muscle points within the
same muscle group or to compare different muscle groups.

Miura et al.\textsuperscript{10} using a multichannel continuous-wave
NIRS system, found regional differences in the oxygenation
of the gastrocnemius muscle during exercise and recovery
performing standing plantar flexion exercises for 2 min (one
contraction/s) with the distal portion having greater deoxy-
genation and tHb changes. This is consistent with the distal
portion having a greater impairment of MBF possibly because
of the higher intramuscular pressure during exercise. Quaresima et al.\textsuperscript{12} investigated vastus lateralis and rectus
femoris VO$_2$ at rest and during maximal voluntary contraction
using a 12 channel continuous-wave NIRS system (0.1 s ac-
quision time). VO$_2$ either at rest or during maximal voluntary
contraction was found to be nonuniform in the 12 mea-
Surement sites over a surface of 8 $\times$ 8 cm$^2$. The results of
these two studies have strengthened the role of NIRS as a
powerful tool for investigating the spatial and temporal fea-
tures of muscle oxygenation changes as well as muscle VO$_2$.
However, optical pathlength heterogeneity in muscle has been
reported.\textsuperscript{13} Only frequency-domain or time-domain photom-
eters (measuring pathlength) can provide accurate measure-
ments of muscle oxygenation/perfusion. Therefore, the results
of this study provide quantitative spatial distribution of the
quadriceps SmO$_2$ and MBF data (Tables 1–3).

Since the NIRS technique is unable to differentiate be-
 tween the amount of oxygen released by hemoglobin (Hb)
and Mb (because their absorption spectra overlap in the near-
infrared range), it still remains controversial the extent of the
Mb contribution to the NIRS measurements obtained even by
using the most advanced devices. However, it must be con-
 sidered that within a given volume of muscle there are differ-
ences in concentration of both Hb and Mb (i.e., Hb is about
1.5 times higher than Mb), and in their binding capacities
(i.e., Hb has four times the oxygen binding sites).\textsuperscript{32} Therefore,
one can estimate Mb mass as a confounding factor at about
20% of the whole NIRS signal. As reported by Richardson
et al., at rest the intramuscular oxygen stores (measured by
the appearance of $^1$HMR$^2$O deoxy-Mb signal during suprasys-
tolic cuff occlusion) begin to decrease after 4 min, and the
maximal Mb desaturation is achieved after 8 min.\textsuperscript{31} Con-
versely, at rest the intramuscular oxygen stores, as measured
by NIRS during suprasystolic cuff occlusion, begin to de-
crease immediately after the beginning of the occlusion and
the maximal desaturation is achieved after 5–6 min.\textsuperscript{32} During
high intensity exercise, Mb typically desaturates to only 50%
of the level attained during cuff occlusion,\textsuperscript{32} and muscle oxy-
genation, as measured by continuous wave NIRS, typically
desaturates to about 90% of the level attained during the cuff
occlusion.\textsuperscript{34} Overall these data would suggest that during
the short quadriceps MVC, the measured value of the vastus lat-
eralis SmO$_2$ reflects predominantly (at least 80%) the
weighted mean of arteriolar, capillary, and venular oxygen
hemoglobin saturation. The remaining can be attributed to the
contribution of myoglobin oxygen saturation. Nevertheless,
more combined $^1$HMR$^2$O and NIRS studies are needed to
clarify the issue of the contribution of Mb to the NIRS signal.
The MBF values found at rest (Table 1) agree with those
obtained by other NIRS and positron emission tomography
studies.\textsuperscript{8,8,29,35}

The combination of NIRS and venous occlusion maneuver
applied during exercise allows for the assessment of the post-
exercise MBF peak which, according to the femoral artery
flow measurements, occurs immediately after the muscle re-
lease. The venous occlusion applied after the end of the exer-
cise would not allow for the measurement of the MBF peak.
The time required to recover the MBF base line value depends
on the duration of the exercise.\textsuperscript{14}

In this study, the venous occlusion was applied 10 s after
the start of the exercise (when tHb was stable) not to measure
MBF\textsuperscript{29} because, as expected, the intramuscular pressure was
high enough to restrict completely MBF to the investigated
muscle group. The contraction intensity was supposed to be
very high (at least 70% of the subject’s own maximal volun-
tary contraction) because MBF was restricted in about 3 s
from the onset of the contraction, as revealed indirectly by the
constancy of tHb in the following 10–15 s of exercise. There-
fore, the venous occlusion, applied while high intensity iso-
metric contraction was maintained, should not affect the per-
fusion of the contracting muscle, the performance of the
exercise, and the muscle metabolic response to the exercise.
The slight increase in tHb, occurring about 20 s after the
beginning of exercise, could be attributable to the partial re-
lease of the intramuscular pressure, in fact, the high intensity
isometric contraction cannot be maintained constant for a pro-
longed time.

The heterogeneous MBF response to the intense isometric
exercise found between the proximal and the distal locations
(Table 3) might be explained by either one or a combination
of the following points: (a) different local blood supply pro-
duced by differences in intramuscular pressure,\textsuperscript{30} (b) diver-
gence of the mechanical activity within the quadriceps, (c)
differences in oxidative metabolic activity, (d) variations in
the vascularity, i.e., distribution of arterial, venous and
capillary vessels; and (e) the recruitment of different fiber
types within the investigated muscle volume. Indeed, MBF
heterogeneity was also found in the quadriceps during dy-
namic and isometric exercise by using positron emission to-
mography imaging.\textsuperscript{17,36} Moreover, also $^3$P-magnetic reso-
nance spectroscopy revealed pH heterogeneity in the tibial
anterior muscle during isometric activity.\textsuperscript{37} This diverse pH
distribution was attributed to intramuscular differences in
blood supply. Sejersted et al.\textsuperscript{30} measuring intramuscular fluid
pressure in three different sites of the vastus medialis, hypo-
thesized that blood flow is first compromised deep in the vastus
medialis muscle where intramuscular fluid pressure is highest
and in general at lower stress or tension in short bulging
muscles with great curvature of the fibers compared with long
sloiner ones.

The positive correlation found between the maximum de-
saturation measured during short very intense isometric con-
truction and the postexercise to pre-exercise MBF ratio (Fig.
4) in the proximal and distal regions confirms the tight cou-
pling between blood flow to muscle and the oxygenation state
of hemoglobin.\textsuperscript{38}

To the best of our knowledge, there are no multichannel
SmO$_2$ data during high intensity isometric exercise. Using a
similar frequency-domain near-infrared oximeter, localized ir-
regularities in MBF and venous oxygen saturation at rest have been recently mapped (22 locations) over an area (18 × 6 cm²) of the calf muscle of healthy subjects and peripheral vascular disease patients. It has been reported that calf MBF of healthy subjects was greater (by about 0.3 mL/100 g/min) in the proximal region compared with the distal region. In our study resting MBF was higher (by about 0.3 mL/100 mL/ min) in two locations of the distal region (D2 and D3) with respect to the corresponding ones of the proximal region (P2 and P3) of the vastus lateralis (Table 1). Conversely, postexercise MBF was lower (by about 50%) in the two locations of the distal region (D2 and D3) than in the corresponding ones of the proximal region (Table 3). The discrepancy between our results at rest and the data of Wolf et al. could be attributable to the different structure and function of the two examined muscle groups of the lower limb.

In conclusion, frequency-domain oximeters are relatively low-cost, noninvasive, powerful tools for investigating the spatial and temporal features of the muscle oxygenation changes as well as the MFB in pathophysiology.

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