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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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Abstract. Muscle blood flow (MBF) and muscle oxygen saturation (SmO_2) were measured at eight locations (four proximal, four distal) over a 4×8 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. [DOI: 10.1117/1.1646417]

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

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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 paradigms[1] and the effects of exercise on muscle oxygenation[2,3] have both been investigated with NIRS. Furthermore, NIRS affords the measurement of regional muscle blood flow (MBF) and oxygen consumption (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 (O_2Hb) 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 ($tHb = O_2Hb + HHb$) and muscle oxygen

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saturation (SmO_2) during isometric, maximal contractions. For this purpose, we used a multichannel NIRS instrument, allowing for simultaneous measurements at eight locations.[18] 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,[19] 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 SmO_2 and in MBF was correctly investigated.

2 Methods

The experiments were carried out on five subjects (age: 24.6 $\pm$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.[18] 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 *P1*, *P2*, *P3*, *P4*) and to the right (for measurements at distal regions *D1*, *D2*, *D3*, *D4*) 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$\times$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 optical fiber (multidistance scheme). The four collection optical fibers are separated by 1.0 cm from each other.

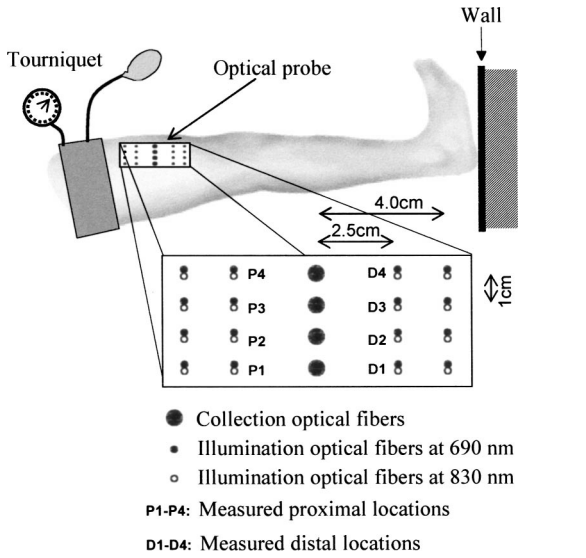

Fig. 1 Illustration of the optical probe placement over an area (4 $\times$8 cm^2) of the right vastus lateralis muscle. Schematic diagram of the optical probe showing the geometrical arrangement of the source and the detector optical fibers.

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[20] at each proximal (*P1*, *P2*, *P3*, *P4*) and distal (*D1*, *D2*, *D3*, *D4*) measured location. (b) The relative measurement of changes in the tissue concentrations of O_2Hb and 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).[21] The multidistance scheme for absolute measurements has been tested *in vitro* on tissue-like phantoms,[22] and *in vivo* on both animal models,[23,24] and human subjects.[25] In particular, absolute measurements of tissue oxygen saturation showed an excellent correlation with the data from cooximetry in the *in vivo* studies.[23,24] 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.[26] 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 O_2Hb and 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 O_2Hb and 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

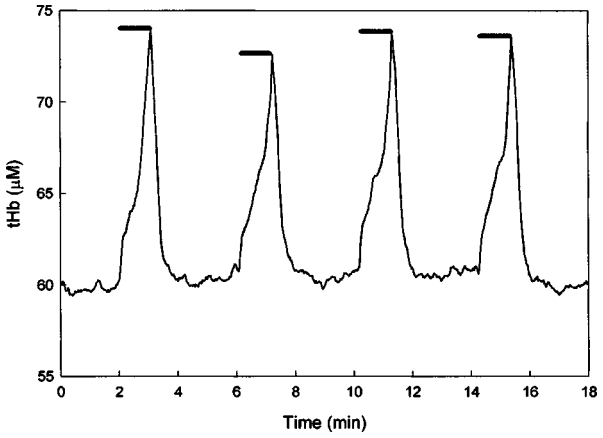

Fig. 2 Representative temporal trace of the tHb measured (using the multidistance approach) at region *P1* (see Fig. 1) of the right vastus lateralis muscle during four consecutive 60 s venous occlusions (data from subject No. 2). MBF at rest was calculated from the maximum rate of increase in the tHb during the first 25 s of venous occlusion. The segments indicate the duration of the inflation of the thigh tourniquet.

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 $4\times 8\ \mathrm{cm}^2$, 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 SmO_2 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 SmO_2 values at the eight measured locations.

The measured value of the vastus lateralis SmO_2 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 drainage 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:

$$\mathrm{MBF}=\frac{1}{C}\left.\frac{d(\mathrm{tHb})}{dt}\right|_{\max}, \tag{1}$$

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 SmO_2 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 SmO_2 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 SmO_2 and MBF values corresponding to each pair of proximal and distal positions. The correlation between changes in SmO_2 and the MBF ratio was expressed by the Pearson's correlation coefficient. Data are presented as mean $\pm SD$. The criterion for significance was $P<0.05$.

Table 1 Right vastus lateralis MBF (mL/100 mL/min) over the eight measurement points ($n=5$). (Data are presented as mean ±SD. For each subject, resting MBF value at each measurement point, is the average of MBF data obtained by four repeated measures.)

	Proximal region			
	P1	*P2*	*P3*	*P4*
At rest	0.5±0.4	0.3±0.1	0.3±0.1	0.3±0.1
After end-exercise	2.0±1.3[a]	2.1±0.6[a]	1.7±0.2[a]	1.6±0.4[a]
At rest	0.6±0.3	0.6±0.3[b]	0.6±0.2[c]	0.5±0.1
After end-exercise	1.8±1.5	1.5±0.5[a,d]	1.4±0.5[a]	1.5±0.7[a]
	D1	*D2*	*D3*	*D4*
	Distal region			

[a] Significantly different from the corresponding value at rest.

[b] Significantly different from *P2* at rest.

[c] Significantly different from *P3* at rest.

[d] Significantly different from *P2* after end of exercise.

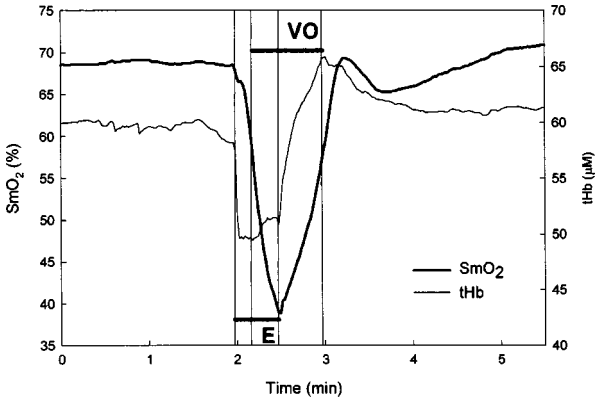

Fig. 3 Representative temporal traces of the SmO_2 and tHb measured (using the multidistance approach) at region *P1* (proximal, detector No. 1) of the right vastus lateralis muscle during isometric, maximal leg contraction (data from subject No. 2). The first and the third vertical bars identify the duration of the exercise (*E*), while the second and the fourth bars indicate the duration of the venous occlusion (VO). The venous occlusion allowed for the measurement of the MBF immediately after the end of the exercise.

3 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 SmO_2 and tHb corresponding to the region *P1* of the right vastus lateralis of subject No. 2 during an isometric and maximal quadriceps contraction is shown in Fig. 3. SmO_2 at rest was about 68% for subject No. 2. At rest, considering all subjects (Table 2), SmO_2 was uniform over the distal region and over the proximal region, except for position *P3* where SmO_2 was significantly higher than in region *P1* ($P<0.05$). Comparing SmO_2 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 2 Right vastus lateralis muscle oxygen saturation (SmO_2, %) over the eight measurement points ($n=5$). (Data are presented as mean ±SD.)

	Proximal region			
	P1	*P2*	*P3*	*P4*
At rest	63.0±5.5	64.6±6.3	71.6±10.1[b]	69.5±5.3
End-exercise	48.8±8.9[a]	43.6±6.4[a]	56.4±14.0[a,d]	56.2±6.7[a,d]
At rest	63.6±9.1	68.3±6.7[c]	66.5±4.2	69.0±6.8
End-exercise	58.4±6.3	58.3±5.2[a,d]	56.1±5.0[a]	61.9±6.8
	D1	*D2*	*D3*	*D4*
	Distal region			

[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.

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

	Proximal region			
	P1	*P2*	*P3*	*P4*
After end-exercise/at rest	5.0±0.8	7.2±2.8	6.0±2.3	5.5±2.4
After end-exercise/at rest	3.0±1.5	3.2±1.7[a]	2.5±1.2[b]	3.2±1.8
	D1	*D2*	*D3*	*D4*
	Distal region			

[a] Significantly different from *P2*.

[b] Significantly different from *P3*.

variability of the SmO_2 and/or the low number of the subjects.

The initial tHb response to the quadriceps exercise was quicker than the SmO_2 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.[30] 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, SmO_2 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. SmO_2 gradually recovered and returned to the pre-exercise value within about 1 min.

At the end of the maximal isometric contraction, SmO_2 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, SmO_2 was uniform over the distal region and over the proximal region, except for position *P3* and *P4* where SmO_2 was significantly higher than in position *P2* ($P<0.05$). Comparing the SmO_2 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 (ΔSmO_2) 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).

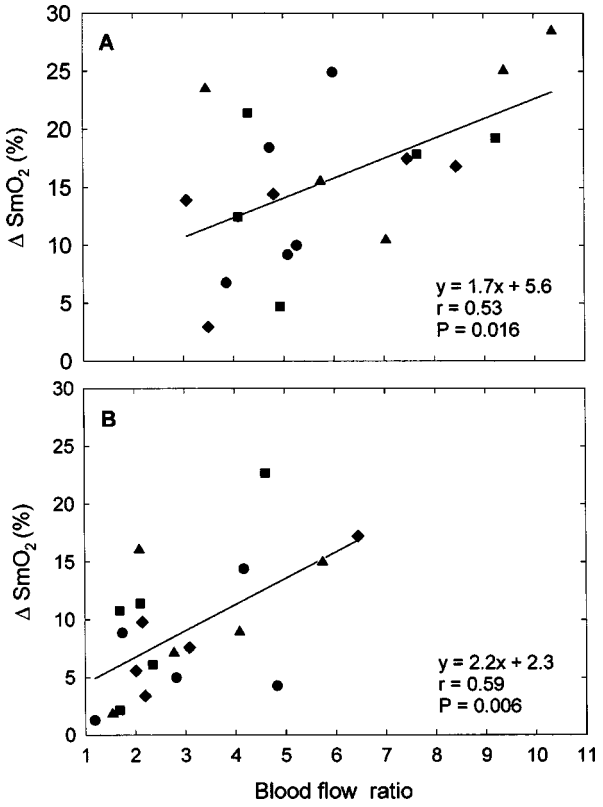

Fig. 4 Relationship between the maximal desaturation (ΔSmO_2) measured during the isometric, maximal leg contraction, and the MBF postexercise to pre-exercise ratio. Panel A refers to the proximal region, while the panel B refers to the distal region of the right vastus lateralis muscle (the circle for *P1* and *D1*; the triangle up for *P2* and *D2*; the square for *P3* and *D3*; the diamond for *P4* and *D4* (subject No. 5).

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 cm^2 surface of the human quadriceps immediately after isometric maximal contraction; second, as expected, MBF after exercise was found significantly increased with respect to base line (except in one location); 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.[2,3] However, the monitoring of single muscle locations is not representative

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 multichannel 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.,[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 deoxygenation 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.[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 acquisition time). VO_2 either at rest or during maximal voluntary contraction was found to be nonuniform in the 12 measurement sites over a surface of $8\times8\ cm^2$. The results of these two studies have strengthened the role of NIRS as a powerful tool for investigating the spatial and temporal features of muscle oxygenation changes as well as muscle VO_2. However, optical pathlength heterogeneity in muscle has been reported.[13] Only frequency-domain or time-domain photometers (measuring pathlength) can provide accurate measurements 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 between 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 considered that within a given volume of muscle there are differences 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).[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}HMRS deoxy-Mb signal during suprasystolic cuff occlusion) begin to decrease after 4 min, and the maximal Mb desaturation is achieved after 8 min.[31] Conversely, at rest the intramuscular oxygen stores, as measured by NIRS during suprasystolic cuff occlusion, begin to decrease immediately after the beginning of the occlusion and the maximal desaturation is achieved after 5–6 min.[32] During high intensity exercise, Mb typically desaturates to only 50% of the level attained during cuff occlusion,[33] and muscle oxygenation, as measured by continuous wave NIRS, typically desaturates to about 90% of the level attained during the cuff occlusion.[34] Overall these data would suggest that during the short quadriceps MVC, the measured value of the vastus lateralis 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}HMRS 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.[8,9,29,35]

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 release. The venous occlusion applied after the end of the exercise 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.[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[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 voluntary 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. Therefore, the venous occlusion, applied while high intensity isometric contraction was maintained, should not affect the perfusion 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 release of the intramuscular pressure, in fact, the high intensity isometric contraction cannot be maintained constant for a prolonged 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 produced by differences in intramuscular pressure,[30] (b) divergence of the mechanical activity within the quadriceps, (c) differences in oxidative metabolic activity, (d) variations in the vascularization, 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 dynamic and isometric exercise by using positron emission tomography imaging.[17,36] Moreover, also ^{31}P-magnetic resonance spectroscopy revealed pH heterogeneity in the tibial anterior muscle during isometric activity.[37] This diverse pH distribution was attributed to intramuscular differences in blood supply. Sejersted et al.,[30] measuring intramuscular fluid pressure in three different sites of the vastus medialis, hypothesized 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 slender ones.

The positive correlation found between the maximum desaturation measured during short very intense isometric contraction and the postexercise to pre-exercise MBF ratio (Fig. 4) in the proximal and distal regions confirms the tight coupling between blood flow to muscle and the oxygenation state of hemoglobin.[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 $\times 6$ cm^2) of the calf muscle of healthy subjects and peripheral vascular disease patients.[9] 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.[9] 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.[9] 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 MBF in pathophysiology.

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