Monitoring muscle metabolic indexes by time-domain near-infrared spectroscopy during knee flex-extension induced by functional electrical stimulation

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Abstract. A noninvasive methodology, combining functional electrical stimulation and time-domain near-infrared spectroscopy (TD-NIRS), is developed to verify whether stroke-altered muscular metabolism on postacute patients. Seven healthy subjects and nine postacute stroke patients undergo a protocol of knee flex-extension induced by quadricep electrical stimulation. During the protocol, TD-NIRS measurements are performed on both rectus femoris to investigate whether significant differences arise between able-bodied and stroke subjects and between patients' paretic and healthy legs. During baseline, metabolic parameters do not show any significant differences among subjects. During stimulation, paretic limbs produce a knee angle significantly lower than healthy legs. During recovery, patients' healthy limbs show a metabolic behavior correlated to able-bodied subjects. Instead, the correlation between the metabolic behavior of the paretic and able-bodied legs allows the definition of two patients' subgroups: one highly correlated ($R > 0.87$) and the other uncorrelated ($R < 0.08$). This grouping reflects the patient functional condition. The results obtained on the most impaired patients suggest that stroke does not produce any systemic consequences at the muscle, but the metabolic dysfunction seems to be local and unilateral. It is crucial to enlarge the sample size of the two subgroups before making these preliminary results a general finding. © *2009 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.3183802]

1 Introduction

Stroke is the third most common cause of mortality and of acquired disability worldwide. Patients with stroke can ac-

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quire significant neurological impairments and also metabolic changes that contribute to poststroke complications.[1] The clinical syndrome resulting from central nervous system (CNS) lesion depends on its location and extent, and the time since it occurred. The clinical and instrumental examinations generally are focused on motor control to check positive symptoms like clonus, increased muscles tone, and released flexor reflexes, and negative symptoms like loss of dexterity, weakness, and the associated muscular changes in terms of atrophy and loss of rheological properties of the muscles. Less attention is oriented to detect peripheral vascular impact on the affected limb, which could play a big part in the motor recovery perspective. These dysfunctions can be linked to microcirculation and/or macrocirculation problems.[2] In the first case, the limb can appear decolorated or cold, and there could be edema or dystrophy. Instead, abnormal macrocirculation can lead to the peripheral vascular disease condition, which is characterized by intermittent claudicating.

Several hypotheses have been suggested to explain the symptoms and complaints of their pathology as a circulatory problem: after stroke, overactivity (hypertonia, dystonia, spasticity, and spastic dystonia) can arise in muscles, and it might hinder blood circulation. In addition, the paresis may lead to a dysfunction of the muscle pump and then to disturbances in macrocirculation and microcirculation. Finally, loss of voluntary muscle control after stroke may lead to atrophy[3] or to abnormal metabolic function.[4] Prolonged voluntary disuse might aggravate further changes toward lowered oxidative metabolism and capillary supply. These metabolic abnormalities in the peripheral tissues can decrease muscle strength and endurance, reducing the possibility to perform the program of rehabilitation exercise that is crucial in the recovery of patients with stroke.

Functional electrical stimulation (FES) is a well established method in the rehabilitation of hemiplegic patients, because it helps the overall rehabilitation process.[5,6] It was demonstrated that treatment based on FES increased muscular strength, enhanced walking ability, and improved motor control, both increasing agonist electromyographic activity and decreasing electromyographic co-contraction ratios.[6–10] Since FES has become an established rehabilitation method for stroke patients, it is crucial to investigate the muscular metabolism of the artificial contraction. It is important to understand its effect on the muscle, and in particular, whether this effect is different between patients and able-bodied (AB) subjects and between the two limbs of the patients.

Among all the noninvasive techniques developed to study tissue hemodynamics of muscles, near-infrared spectroscopy (NIRS) seems to have good potential.[11,12] NIRS provides high temporal resolution and relatively low spatial resolution without having the high costs and poor mobility of magnetic resonance imaging and positron emission tomography.

Few studies exist on the application of NIRS to investigate the metabolism of stroke patients, but they mostly involve chronic patients in which there is an important muscle atrophy affecting the muscle properties (a transformation from oxidative to nonoxidative fibers caused by the disuse) and muscular vascularization.[13,14] In particular, Jigjid et al. analyzed the changes in oxygenation during a passive leg movement and during the recovery phase after the movement on chronic stroke patients. They demonstrated that there was a significant difference in the concentration changes of the oxygenated hemoglobin between the muscles of the paretic and nonparetic sides.[13] The degree of the changes of the O_2Hb depended on the level of motor recovery after stroke and on the time passed after the stroke event. Indeed, subjects with good motor recovery showed less difference in the O_2Hb level between the paretic and nonparetic sides of the muscle.

Our study arises from the hypothesis that stroke produces an altered muscular metabolic environment, not only on chronic but also in postacute patients, and that the use of time-domain NIRS to measure metabolic parameters during a repetitive artificial contraction induced by FES could be very helpful in understanding the muscular consequences of pathology.

In particular, the experiments described in this study follow two questions. 1. What is the impact of a cerebral vascular pathology provoking primarily neurological impairments, such as stroke, on the muscular metabolism? To answer this first question, a comparison between the muscular metabolisms measured locally on the paretic and healthy limb of patients and on both the legs of AB subjects of similar age was carried out. In particular, we chose to measure the muscular metabolism during a contraction induced by FES, because FES assured a greater repeatability of the exercise with respect to a good voluntary motor task that sometimes is difficult to obtain mainly on these patients. 2. Are there any correlations between the functional indexes used in clinics to evaluate the patient recovery [e.g., the motricity index (MI)] and the muscular metabolism measured during an artificial contraction? In this case, the metabolic parameters could help in monitoring the rehabilitation and probably in choosing the best treatment able to optimize and speed up the recovery.

2 Methods

2.1 Experimental Setup

2.1.1 Functional electrical stimulation

Quadricep muscles were stimulated by adhesive rectangular surface electrodes. The knee joint angle was measured by an electrogoniometer, which comprised two plastic bars attached to the thigh and shank, and a linear potentiometer fixed on the joint. This device was interfaced with the PC by means of an A/D board with a sample rate of 20 Hz. A current-controlled eight-channel stimulator, RehaStim Pro™ (Hasomed GmbH, Germany), was used during the experiments. The entire setup was connected to a Linux-PC running Matlab/Simulink™ for data acquisition and for control of the stimulation device.

2.1.2 Time-domain near-infrared spectroscopy

A dual-wavelength (690/820 nm) multichannel time-resolved system for NIRS was used. The system was based on picosecond laser sources, fiber optics switches and fused splitters for light delivery, homemade fiber bundles and multianode photomultipliers for light collection, and time-correlated single photon counting electronics for data acquisition.[15] TD-NIRS measurements were performed simultaneously on the left and right legs. The optical probe (consisting of a few injection and detection optic fibers at a relative distance of 2 cm) was placed over the rectus femoris, in between the FES electrodes, at about 10 cm from the upper edge of the patella.
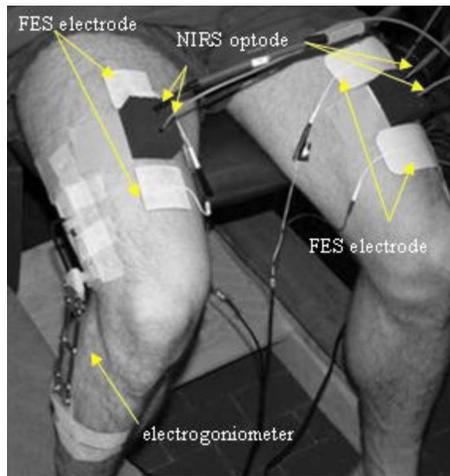
Fig. 1 The experimental setup.

Custom-made fiber holders were designed to keep fibers normally to the skin by black rubber pads. Holders were kept in place on the muscle of the subject by biadhesive tape (see Fig. 1). We avoided Velcro® bands that can cause limb occlusion or compression during exercise.

Simultaneous estimate of reduced scattering coefficient and absorption coefficient for each source-detector couple was achieved by best fitting of time-resolved reflectance curves with a standard model of diffusion theory.[16] To enhance the contribution from deep layers and to remove possible disturbances caused by superficial adipose layers, a correction method based on the use of late time windows (1750 to 2500 ps) was also applied.[17] Taking the assumption that deoxy- and oxyhemoglobin (HHb and O_2Hb, respectively) are the main chromophores contributing to absorption, their concentrations are easily derived by using the knowledge of the extinction coefficient.[18]

An important issue to notice is that the NIRS technique is unable to distinguish between the amounts of O_2 released by hemoglobin or myoglobin, because the optical spectra of the two chromospheres are overlapped. However, within a given volume of muscle, the concentration of hemoglobin is about 1.5 times greater than the concentration of myoglobin, and hemoglobin has four oxygen binding sites compared to one binding site of myoglobin.[19] Therefore, it was estimated that the contribution due to myoglobin affects less than 20% of the oxygen response.[20]

The NIRS signals, electrical stimulation, and knee angle were properly synchronized.

2.2 Subjects

Seven male AB subjects and nine male stroke patients took part in the study. Mean age was 53.4 years (range 29 to 72 years) for AB subjects and 55.6 years (range 25 to 72 years) for patients. Mean body mass index was 23.4 kg/m^2 (range 20.5 to 26.8 kg/m^2) for AB and 22.8 kg/m^2 (range 18.7 to 26 kg/m^2) for patients. Only two of the healthy subjects performed a frequent physical activity (more than two days a week), while the others led a sedentary life.

All the stroke patients were recovered in the clinic and underwent about 3 h a day of rehabilitation. The rehabilitation performed with therapists included stretching, muscular conditioning with active or passive motility, and exercises to recover trunk control, standing position, and walking. The sequence and composition of exercises was customized for each patient. The group was very heterogeneous in terms of their level of recovery after the stroke. Conditions for the study were that the patients had to be collaborative, able to understand simple instructions, be without any joint rigidity, and have an Ashworth <2 in all the lower limb muscles.[21] No patients had evidence of peripheral vascular disease. Only P5 has diabetes mellitus. All the patients except P7 were postacute, i.e., less than 6 months had passed after the stroke. Table 1 reports details on the clinical data of the stroke patients included in the study. In particular, the values of MI obtained by the paretic lower limb of the patients are shown. MI is a clinical index used to evaluate the mobility of the ankle, knee, and hip joint during voluntary movements performed against gravity or against an external resistance.[22] Finally, the thickness of the adipose tissue over all the investigated sites was less than 5 mm for all subjects.

Written informed consent was obtained from all subjects. The study was approved by the ethical review board of Villa Beretta Rehabilitation Centre (Costamasnaga, Italy).

2.3 Protocol

The test subject was seated on a bench that allowed the shanks to swing freely. Once attached to the stimulation electrodes, the stimulation current amplitudes for the quadriceps were selected. For all the subjects, the stimulation pulse width used was 400 μs and the stimulation frequency was 20 Hz, while the current amplitude was selected individually for each leg. Fixing the pulse width at 400 μs, the current amplitude was increased in steps of 5 mA up to the level able to yield a tolerated massive contraction of the rectus femoris with a complete extension of the leg when it was possible. Once the optodes were attached on the rectus femoris (see Fig. 1), the test begun. Each subject performed two trials, one per each leg. The single trial lasted 8 min: during the initial 30 s, the subject was not stimulated at all (baseline period); then the quadriceps was stimulated for a total of 225 s, in which 15 flex-extension movements were induced by FES (the stimulation was on for 10 s and off for 5 s); in the remaining part (225 s), the stimulation was off. TD-NIRS measurements on both legs were carried out during the whole trial (8 min) with a sampling time of 0.2 s.

The FES knee flex-extension exercise was intended as a fatigue-inducing exercise, and the knee angle was the measure of this fatiguing effect.

2.4 Data Processing

The trajectories of the concentration of HHb and O_2Hb were analyzed. Then the oxygen saturation [$SO_2 = O_2Hb/(HHb + O_2Hb)$] and the total hemoglobin concentration (tHb $= HHb + O_2Hb$) were derived.

2.4.1 Initial baseline period

For each subject, the metabolic behavior of both legs was analyzed. The mean value of HHb, O_2Hb, SO_2, and tHb ob-

Table 1 Details on the patients included in the study. The range of MI is comprised between 0 and 100, where 100 means that the functionality is completely recovered. MI_{in} and MI_{out} are the values measured when the patient was admitted and dismissed from the hospital. The MI_{test} is the estimated value of MI computed from the linear interpolation between MI_{in} and MI_{out}.

Subject	P1	P2	P3	P4	P5	P6	P7	P8	P9
Age [years]	69	67	72	50	61	49	70	52	25
Days between ictus and hospital admission	73	16	133	75	12	12	1191	20	151
Hemorrhagic (H) or ischemic (I)	I	I	I	I	H	H	I	H	H
Paretic side: left(L)/right (R)	L	L	L	R	L	L	R	R	R
Days between hospital admission and test day	40	8	21	32	21	35	5	38	4
Days between test day and patient discharge	7	11	18	26	11	114	22	3	9
MI at admission MI_{in}	49	76	75	43	57	15	47	47	76
MI at discharge MI_{out}	49	100	75	53	75	43	59	59	100
MI at the test day MI_{test}	49	86	75	49	69	22	49	58	83
Body mass index [kg/m^2]	23.8	25.8	22	26	23.7	18.9	24.8	18.7	21.6
Peripheral vascular problems or diabetes	no	no	no	no	diabetes mellitus	no	no	no	no

tained in the first 30 s were computed in the first trial to avoid possible effects of the sequence of exercise on the baseline value.

2.4.2 Functional electrical stimulation exercise period

We computed the angular amplitude of each leg extension movement as the difference between the maximum knee angle reached in the 10 s in which the stimulation was on, and the mean knee angle value maintained during the 5 s previous to each stimulation onset. Then, for each subject we computed the mean angular amplitude obtained during 1 to 5, 6 to 10, and 11 to 15 flexion-extension movements, called A_1, A_2, and A_3, respectively.

In addition, during the FES exercise, the muscle oxygen consumption (VO_2, expressed in units of ml $O_2 / 100$ g/min) was calculated, according to the method previously described in Refs. 23 and 24. In particular, we measured VO_2 in the flex-extensions 8 to 15, when the tHb was fairly constant. VO_2 was computed from the slope of the decrease in O_2Hb during the last 8 s of each 10 s flex-extension.

2.4.3 Recovery period

The metabolic time courses were filtered with a Savitzky-Golay filter with a time window of 75 s, and normalized to the corresponding initial values of the recovery period. For all the metabolic parameters, a normality area limited by the quartiles of the recovery trajectories obtained by AB subjects was computed.

To evaluate the metabolic recovery of patients, the HHb, O_2Hb, SO_2, and tHb trajectories obtained by each patient during the recovery phase were compared to the correspondent normality areas. In particular, correlation coefficients (R)

obtained between the recovery trajectories measured on each patient of the group, and the mean trajectories computed averaging the AB subject behavior, were calculated. Thus, R was calculated on 1125 samples (225 s at 5 Hz).

2.5 Statistics

A statistical analysis was performed using Matlab/Statistical Toolbox, (Mathworks, Natick, Massachusetts) on the following variables:
   • mean value of HHb, O_2Hb, SO_2, and tHb obtained during the initial baseline period
   • angular amplitudes A_1, A_2, and A_3
   • the VO_2 obtained during the FES exercise.
After verifying that all parameters were not normally distributed (Kolmogorov Smirnov $p < 0.05$), a Kruskal Wallis ($p < 0.05$) nonparametric intersubjective statistical test was carried out to verify the significant differences among the following groups: AB limbs (ABL), patient healthy limbs (PHL), and patient paretic limbs (PPL). For significant effects of each Kruskal Wallis, Dunn-Sidak *post-hoc* tests were performed ($p < 0.05$) to determine which pairs of effects were significantly different.

3 Results

The trajectories of the concentration of HHb, O_2Hb, SO_2, and tHb were analyzed. Figure 2 shows a comparison of the time courses of ΔHHb, ΔO_2Hb, ΔSO_2, and ΔtHb that are the variations with respect to their mean baseline value, in the stimulated and the resting leg of an AB subject and on a subject with stroke (P1). For P1, the stimulated leg is the paretic one. During the whole trial the resting limb maintains a stable condition both in the AB subject and in the patient. In the baseline period (i.e., first 30 s of the protocol), ΔHHb,
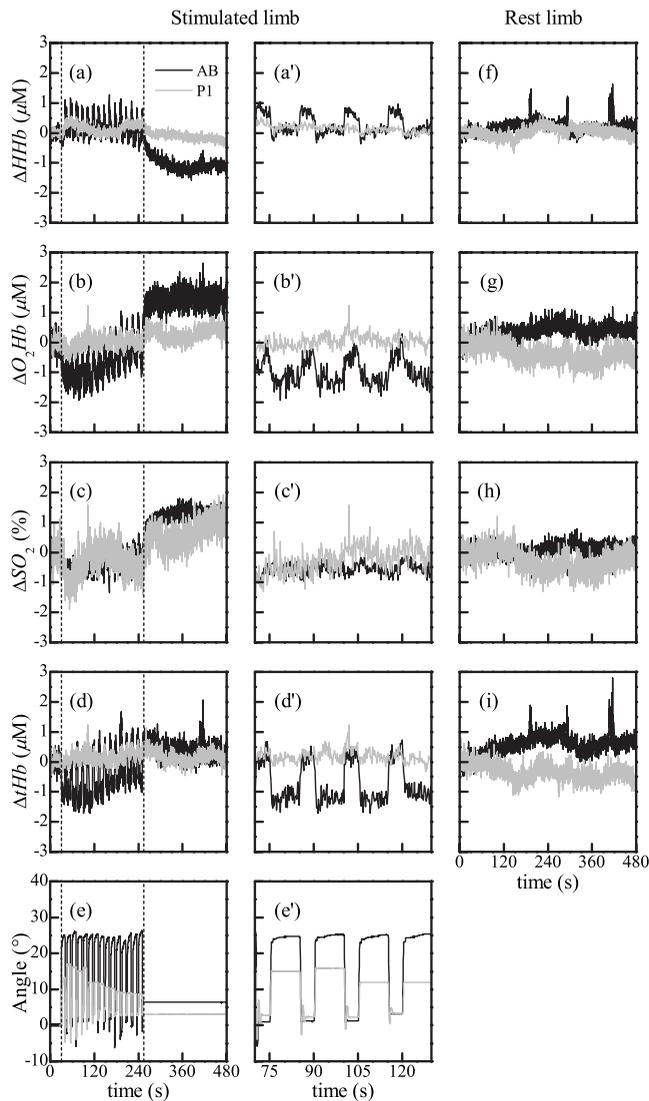
Fig. 2 Example of the typical curves of one healthy subject (black line) and the stroke patient P1 (gray line). For P1, the stimulated limb is the paretic one. Panels (a) through (e) report respectively the ΔHHb, ΔO_2Hb, ΔSO_2, ΔtHb and knee angle obtained by the stimulated leg. (a′) through (e′) show a magnified view of panels (a) through (e) during three movements of the FES-induced exercise. (f) through (i) report the metabolic time curves obtained on the limb at rest.

ΔO_2Hb, ΔSO_2, and ΔtHb were rather stable for all the AB and patient limbs. During the FES application, a spiking oscillating behavior was observed on the AB stimulated limb in all the time curves [Figs. 2(a′) to 2(d′)]. This behavior is probably associated with the rapid muscle contraction that induces a sort of occlusion on the tight. This trend is less evident in the paretic leg of the patient. After the end of the FES exercise (after 255 s), in the stimulated legs of the healthy subjects a slow decrease in ΔHHb [Fig. 2(a)], a gradual increase in the ΔO_2Hb [Fig. 2(b)], and ΔSO_2 [Fig. 2(c)] were noticed; ΔtHb remained quite stable [Fig. 2(d)]. These trends indicated a recovery of the oxygenated blood substituting the deoxygenated one maintaining an overall equilibrium. In paretic limbs, similar results can be qualita-

tively observed, but all the variations were less apparent than in AB subjects [Figs. 2(a)–2(d)]. The comparison between the functional behavior of one leg of one AB subject and the paretic leg of P1 is reported in Figs. 2(e) and 2(e′), where the knee angle produced by the stimulation of the quadriceps is shown. The maximum extension reached by the patient is less than half of the one produced by the AB subject. Observing the time profile of the angle during the exercise, it is clear that the exercise seems to be more fatiguing for the patient paretic limb than for the AB subject.

3.1 Initial Baseline Period

The metabolic behavior obtained in the baseline period was analyzed. In Table 2 the absolute mean baseline values of HHb, O_2Hb, SO_2, and tHb are reported. These baseline values are important to define the initial conditions and to understand if there are significant differences in the basal blood flow of the three groups, ABL, PHL, and PPL (Fig. 3). Comparing patients' initial value ranges and ABL ones, similar variability was observed, confirmed by the Kruskal Wallis test. The initial values of HHb and O_2Hb were widely spread among patients, even if between the two legs of each patient similar values were obtained (Table 2). However, it would be important to notice that the PPL always started from lower hemodynamic values; this means that a slight lower blood volume was available in the paretic leg.

An overall comparison of the two leg values by statistical analysis confirmed that there were not differences between the two lower limbs of the patients.

3.2 Functional Electrical Stimulation Exercise Phase

In Table 3 the angular amplitudes obtained in the beginning (A_1), middle (A_2), and end (A_3) of the FES exercise are reported. The patient P7 was excluded from the analysis because during the exercise he did not produced any angular movement with the paretic limb. Therefore, it was not possible to assert that the FES exercise was really fatiguing or at least that it had a metabolic significant impact on the patient. The amplitude of the knee angle obtained by the three groups is shown in Fig. 4. The angular amplitude produced by PPL was at least half of the one produced by ABL and PHL, as shown in Fig. 4, in all three phases of the movement $(A_1, A_2,$ and $A_3)$. Instead, the PHL group behaved similarly to the ABL: no significant differences were found between PHL and ABL groups.

The VO_2 values obtained by each patient, and the median and quartiles produced by ABL, are reported in Table 4. For patients P1 and P6, we got inconsistent (negative) values. All the patients except P4 consumed similar amounts of O_2 with the paretic and not paretic leg; P4 instead presented a greater VO_2 for PPL than for PHL. As shown in Fig. 5, the PHL and PPL groups present a greater dispersion of VO_2 with respect to ABL. The Kruskal Wallis test did not show any significant difference among the three groups.
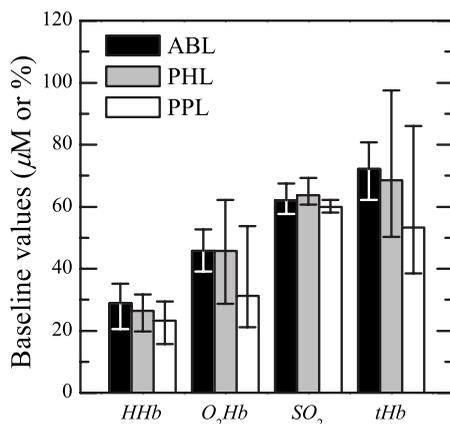
3.3 Recovery Phase

The hemodynamic behavior obtained by each AB subject during the recovery phase is shown in Fig. 6 [ΔHHb in Fig. 6(a), ΔO_2Hb in Fig. 6(b), ΔSO_2 in Fig. 6(c), and ΔtHb in Fig. 6(d)]. It is noticeable that during recovery the right (solid line)

Table 2 Mean values of the HHb, O_2Hb, SO_2, and tHb measured during the first 30 s of the protocol [baseline period (BL)]. Concerning AB subjects, only the median value and the quartiles of the parameters obtained by the two limbs of the whole group are shown.
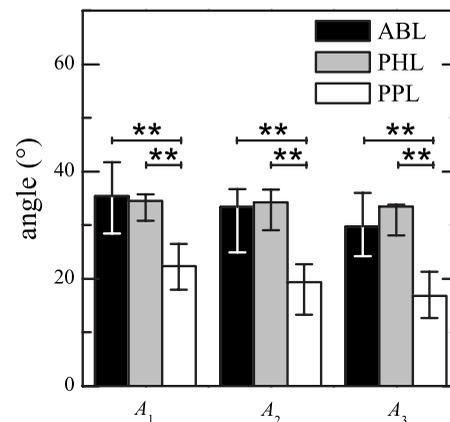
Subject	BL HHb [μM]		BL O_2Hb [μM]		BL SO_2 [%]		BL tHb [μM]	
	PHL	PPL	PHL	PPL	PHL	PPL	PHL	PPL
P1	20.8	12.1	29.7	17.5	58.8	59.2	50.4	29.6
P2	24.2	21.9	25.7	22.2	51.5	50.4	49.9	44.1
P3	35.1	44.8	63.6	55.2	64.5	55.2	98.7	100.1
P4	28.7	16.9	48.6	24.6	62.9	59.4	77.3	41.5
P5	8.2	7.02	20.7	16.1	71.7	69.7	28.9	23.1
P6	17.0	24.7	42.9	37.8	71.7	60.5	59.9	62.5
P7	27.1	15.8	26.0	22.7	49.0	59.1	53.0	38.5
P8	39.0	34.9	61.8	53.2	61.3	60.4	100.8	88.1
P9	30.6	27.7	66.4	57.6	68.5	67.5	97.0	85.3
Median ABL	28.8		45.8		62.0		72.2	
Quartiles ABL	[20.5 to 35.2]		[39.1 to 52.7]		[57.7 to 67.4]		[62.1 to 80.8]	

and left (dotted line) leg of AB subjects present a similar hemodynamic behavior. The results obtained by the AB subjects at the end of the FES-induced exercise allowed us to define a standard behavior in the four metabolic parameters. An increase of ΔO_2Hb and ΔSO_2 is associated with a correspondent decrease of ΔHHb, followed by a plateau state for the three parameters. Instead, ΔtHb remained quite stable, especially after the first few seconds of recovery. This behavior is representative of a newly gained metabolic equilibrium. To evaluate the metabolic recovery of patients, the trajectory obtained by each patient during the recovery phase was compared to the normality area (Figs. 7 and 8). From a qualitative

comparison between the metabolic recovery of patients paretic limbs and the normality areas, it was possible to identify two classes of patients. The first group (called group 1) showed a metabolic trend in the paretic leg similar to the one obtained by the AB subjects [Figs. 7(a)–7(d)]. The paretic limb of the second group (called group 2) is completely different from the mean behavior identified on ABL [Figs. 8(a)–8(d)]. The ΔO_2Hb, ΔHHb, and ΔSO_2 measured on the paretic limb of group 2 remained quite stable with the values

Fig. 4 The knee-angle amplitude obtained by the three groups of limbs. The median and quartiles are referred to the first five (A_1), second five (A_2), and third five (A_3) movements, respectively. The extremities of the lines over the bars indicate that the Dunn-Sidak posthoc test showed a significant difference between the two groups. * means that $p < 0.05$; ** means that $p < 0.01$.

**Fig. 3** Median and quartiles of the baseline absolute value of the HHb, O_2Hb, and tHb (μM) and SO_2 (%).

Table 3 A_1, A_2, and A_3, obtained during the FES exercise. The angle obtained by the healthy limb of P3 was not available (NA) because of problems with the electrogoniometer during the test. However, a visual inspection of the experiment showed us that the patient was moving the leg during the FES exercise.

| Subject	A_1 [deg] PHL	A_1 [deg] PPL	A_2 [deg] PHL	A_2 [deg] PPL	A_3 [deg] PHL	A_3 [deg] PPL
P1	7.0	13.5	13.2	7.7	17.8	5.9
P2	31.1	21.1	32.5	18.0	32.5	16.6
P3	NA	16.0	NA	10.3	NA	9.8
P4	36.7	26.1	41.6	22.3	43.1	20.7
P5	34.5	33.0	36.6	30.9	33.5	27.8
P6	34.8	18.6	36.6	14.3	33.7	13.7
P7	12.3	0	9.5	0.6	11.5	−0.1
P8	37.1	27.6	34.2	24.0	33.9	23.2
P9	30.6	23.6	25.7	20.7	23.7	17.2
Median ABL	35.4		33.4		29.8	
Quartiles ABL	[28.4 to 41.7]		[25.0 to 36.7]		[24.2 to 36.0]	

they have at the end of the FES phase, or they have an opposite trend with respect to the ABL. The end of the exercise is characterized by the absence of blood recruitment in the paretic limb during recovery. It is noteworthy that the behavior of all the healthy legs of the patients in both groups was very similar to the behavior of ABL [Figs. 7(e)–7(h) and Figs. 8(e)–8(h)].

The correlation coefficients (R) obtained between the recovery trajectory measured on each patient and the mean trajectory obtained on AB subjects is reported in Table 5. Since tHb recovery did not show any significant trend on healthy subjects, we decided that correlation analysis was not significant for tHb. To support the significance of correlation analy-
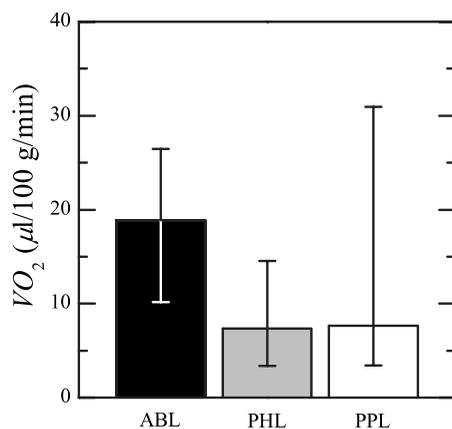
Fig. 5 The median and quartiles of VO_2 obtained by the three groups of limbs.
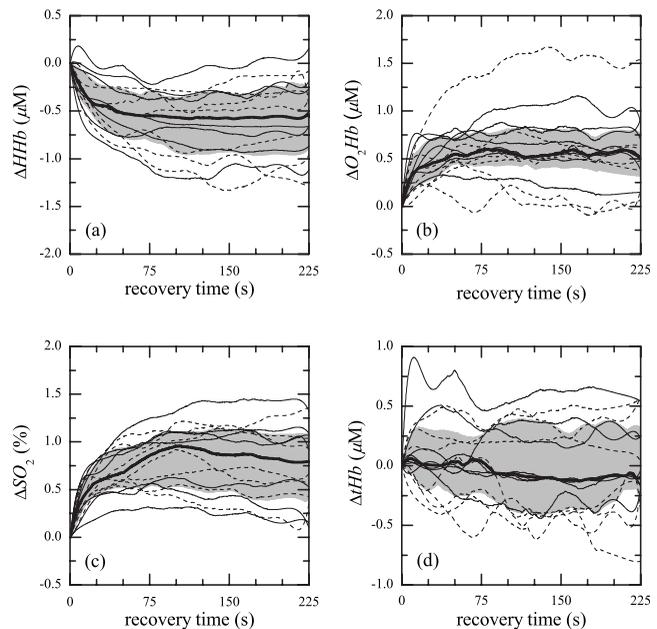
Fig. 6 (a) ΔHHb, (b) ΔO_2Hb, (c) ΔSO_2, and (d) ΔtHb obtained by all the AB subjects during the recovery phase are shown. Solid and dotted lines indicate the mean trajectories obtained by the right and left lower limbs, respectively. The bold black line indicates the mean trajectories obtained by AB subjects, and the gray area is the normality area delimited by the quartiles of the AB trajectories.

Table 4 VO$_2$ obtained during the FES exercise.

Subject	VO$_2$ [μl O$_2$/100 g/min] PHL	PPL
P1	–7.3	–5.1
P2	7.6	6.3
P3	5.4	7.4
P4	7.2	25.0
P5	7.6	7.9
P6	–2.8	–5.8
P8	55.0	48.9
P9	35.2	57.4
Median ABL	19.0	
Quartiles ABL	[10.0 to 26.0]	

Fig. 7 The trajectories of the metabolic indexes [ΔHHb (a) and (e), ΔO$_2$Hb (b) and (f), ΔSO$_2$ (c) and (g), and ΔtHb (d) and (h)] obtained by group 1 during the recovery phase are compared with the same trajectories obtained by AB subjects. In each panel the gray area is the normality area already defined in Fig. 6.

sis, the median value of the correlation obtained for each healthy subject with respect to the mean trajectory obtained on all the AB subjects was computed. It was 0.91, 0.82, and 0.91 for the deoxygenated, oxygenated hemoglobin, and the oxygen saturation, respectively.

The results confirmed that all the PHL produced a recovery trajectory always similar to the mean curve measured on the AB subjects, because the mean R values were always larger than 0.7 except for patient P1.

The correlation coefficient R obtained for all the PPL of group 1 was always greater than 0.87, implying a behavior similar to the AB group. The correlation coefficient R obtained for all the PPL of group 2 was in average 0.08 and often negative (Table 5), implying that PPL of group 2 had the opposite trend of the mean curve obtained on AB subjects.

4 Discussion

An experimental protocol was developed to analyze the metabolic response measured by TD-NIRS during a knee flex-extension movement induced by FES in age-matched control subjects and postacute stroke patients. The muscular metabolic indexes were analyzed in three different phases of the trials: at rest, i.e., during the initial baseline period, during the FES-induced exercise, and during the final recovery phase. Control subjects showed a very comparable behavior in all the metabolic parameters during the protocol, especially in the recovery phase. In particular, the two limbs did not differ. This is the first outcome of the present work, indicating that the design of the protocol is reliable and repeatable.

The existing studies on blood flow and on muscle metabolism at rest on patients with stroke showed conflicting results. Redish et al.[25] observed higher basal blood flow in the paretic limb in comparison to the healthy limb; Goldberg, Skowland,
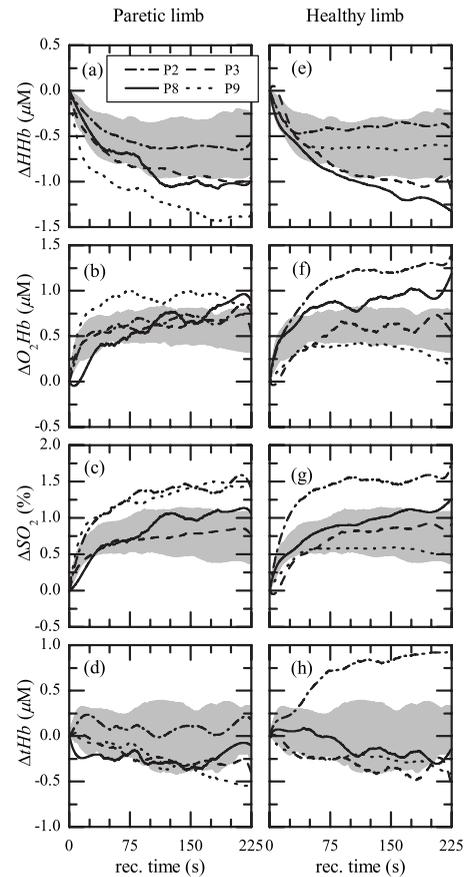
and Katke[26] reported no changes between the two sides. A more recent study showed that resting blood flow was significantly reduced in the paretic leg of chronic stroke patients.[1] Differences between the results of all these studies can be attributed to the lack of comparability of the patient population analyzed. This is a very common problem when considering patients with stroke. All the patients who participated in our study were in a postacute stage (1 to 6 months after the stroke), and we had no differences among the resting values of HHb, O$_2$Hb, SO$_2$, and tHb measured on the two legs (Fig. 3). In addition, it could have been expected that muscle temperature is different between the impaired and unimpaired leg, and this could influence the blood flow at rest. It was reported that immediately after ischemic stroke, temperature is frequently elevated in the affecting limb for an undefined period before showing predominant signs of coolness or normothermia.[1] However, the baseline conditions are not different in our measures.

During the FES-induced exercise, VO$_2$ demonstrated to be a parameter unable to differentiate the three groups because of a great dispersion of values. Indeed, the main hypothesis required by the method adopted for the estimation of VO$_2$,[23,24] i.e., to have a constant tHb, is not completely respected during the induced exercise, because FES does not induce a complete

Table 5 Correlation coefficients (R) computed between the recovery trajectory of ΔHHb, ΔO_2Hb, and ΔSO_2 obtained by each patient, and the mean recovery trajectories of the ΔHHb, ΔO_2Hb, and ΔSO_2 obtained by AB subjects. R is reported both for the healthy (PHL) and paretic limb (PPL) and trajectories obtained by the group 1 (high MI) and group 2 (low MI). All the R obtained were significant (p $\leqslant 0.01$).

		PHL			PPL		
	ID	ΔHHb	ΔO_2Hb	ΔSO_2	ΔHHb	ΔO_2Hb	ΔSO_2
GROUP 1	P8	0.86	0.87	0.84	0.88	0.73	0.84
	P3	0.91	0.88	0.92	0.93	0.92	0.88
	P9	0.98	0.72	0.93	0.91	0.94	0.88
	P2	0.73	0.89	0.94	0.94	0.90	0.92
	Median	0.84	0.87	0.91	0.91	0.87	0.88
GROUP 2	P6	0.93	0.77	0.96	0.43	−0.66	−0.50
	P1	0.90	−0.20	0.44	0.73	−0.17	0.24
	P4	0.98	0.94	0.95	−0.42	−0.41	−0.47
	P5	0.97	0.83	0.91	−0.41	−0.57	−0.66
	Median	0.95	0.58	0.81	0.08	−0.45	−0.35

occlusion. The same reason could explain the fact that for P1 and P6, the estimated value of VO_2 is negative (see Table 4). Indeed, the method used to estimate VO_2 was not applicable for two patients.

The functional performance (knee angle induced by the quadricep stimulation) produced by PPL was always significantly lower than the one produced by PHL and ABL. In addition, a greater fatigue was produced by PPL with respect to both ABL and PHL; indeed, the greatest decrease trend from A_1 to A_3 was produced by PPL (Fig. 4). This behavior during the exercise implies that though the two groups did not consumed different amounts of O_2 to produce the exercise, the PPL showed a less efficient mechanism of muscular excitation/contraction, resulting in a lower knee angle of flex extension and in a greater fatigue effect. This conclusion can be supported by noting that the energy cost can be estimated by VO_2, while the produced work is proportional to the knee angle, the anthropometric data being similar for all subjects.

The analysis of the ΔHHb, ΔO_2Hb, and ΔSO_2 during the recovery after the FES exercise showed that the local muscular metabolism of all the healthy limbs of patients was similar to the metabolism of ABL. R computed between the mean recovery trajectories of AB subjects and the metabolic trajectory of the healthy limb of each patients was on average greater than 0.58 (Table 5). On the basis of the hemodynamic parameters measured during the recovery phase on the paretic legs, it was possible to identify two classes of patients. Group 1 produced a metabolic trend similar to the one obtained by healthy subjects ($R > 0.87$). The metabolic behavior of group 2 was different from ABL ($R < 0.08$).

The behavior of the muscular metabolic parameters measured on the paretic legs seems to be correlated to the patient MI (question 2). Patients of group 1 had a muscular metabo-

lism of the impaired leg similar to the AB one, and all of them are characterized by $MI \geqslant 58$, which means that they have mostly recovered movement functionality. Instead, the impaired legs of patients in group 2 presented a very low correlation coefficient R with AB ones, and in particular, all these patients presented a $MI < 58$ except for P5. Although he presented a MI equal to 69, he was inserted in group 2 because of his metabolic behavior. This patient has diabetes mellitus, and this could have affected the metabolic results. Therefore, it would be possible to assert that a functional recovery seems to be correspondent to a physiological muscular metabolic regulation.

Since we found this difference on the muscular metabolism of the impaired legs of the two groups of patients, we carried out a nonparametric Mann-Whytney-Wilcoxon statistical test between group 1 and group 2 on the following parameters: A_1, A_2, A_3, and VO_2, but no significant differences were found. Therefore, it is noteworthy that the two groups of patients moved their knees similarly during the tests; hence the local functionality induced by FES did not vary, though the two groups were functionally different (according to the MI).

The fact that only the impaired legs of group 2 have a metabolism different from AB ones suggests that the most functionally impaired patients have no systemic consequences at the muscles, but the problem seems to be local and unilateral (question 1). Before making this deduction a general finding, it has to be confirmed by performing localized measurements of blood flow, which cannot be derived simply by the derivative of the tHb, because this hypothesis is correct only during venous occlusion and this was not the case in our experiments. Localized measurements of blood flow could be crucial to investigate which are the potential mechanisms responsible for the observed unilateral problem. Some previous

Paretic limb   Healthy limb
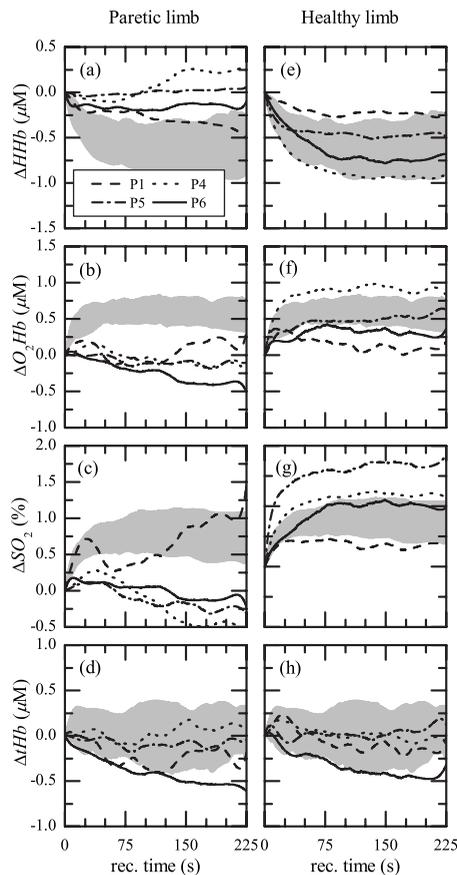
Fig. 8 The trajectories of the metabolic indexes [ΔHHb (a) and (e), ΔO_2Hb (b) and (f), ΔSO_2 (c) and (g), and ΔtHb (d) and (h)] obtained by group 2 during the recovery phase are compared with the same trajectories obtained by AB subjects. In each panel the gray area is the normality area already defined in Fig. 6.

studies suggest vascular dysfunctions such as enhanced sensitivity to endogenous vasoconstrictor agents,[27] changed histochemistry and morphology of the vascular network itself,[28,29] and altered autonomic function.[30] However, the relative contribution of each of these factors is still unknown. Furthermore, the unilateral impairment could be due to muscle atrophy,[31] but this is typically more frequent in chronic and not in postacute stroke patients.

5 Conclusions

We develop a noninvasive powerful methodology to monitor hemodynamic response during muscle contraction induced by FES in healthy and stroke subjects.

The method proposed in this study seems to be a good tool in the understanding of the muscular metabolic consequences of the stroke. A better investigation is needed, but the use of the NIRS in association with FES can give us a quantitative measure of the local muscle metabolism associated with a fatigue-inducing exercise. For instance, the metabolic indexes during the recovery after an exercise could be used to understand if the functional recovery produced by the rehabilitation and measured by other standard clinical tests such as the MI correspond also to a recovery or reorganization of the metabolic functions.

An improved measurement of hemodynamic indexes may lead to better screening, treatment plan, and functional recovery prognosis, as well as to enriched fundamental understanding of muscle function both during voluntary or electrically induced exercises. These preliminary results suggest that it is important to enlarge the study to include a larger number of stroke patients properly shared according to their muscular functionality to have a more precise metabolic discrimination.

Further work is in progress to see if the method could become a tool able to enrich the evaluation criteria of the patient condition before and after rehabilitation, to plan appropriate exercise programs, and to increase the possibility to predict the outcome.

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