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Preliminary study of muscle contraction assessment by NIR spectroscopy

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ABSTRACT
NIR spectroscopy allows monitoring of muscle oxygenation and perfusion during contraction. The knowledge of modifications of blood characteristics in body tissues has relevant clinical interest. A compact and reliable device, which makes use of two laser diodes at 750 and 810 nm coupled with the skin surface through optical fibres, was tested. NIR and surface EMG signals during isokinetic contractions were studied. A set of parameters was analysed in order to obtain information about metabolic modifications during muscle fatigue.

1. INTRODUCTION
The muscle fatigue is defined as a biological event characterised by the incapacity to maintain a fixed level of force during a prolonged exercise. The endurance, defined as the resistance to a sustained effort, and the endurance time, defined as the resistance time performing a series of fatiguing contractions, were chosen as indicators, in order to quantitatively determine the amount of fatigue [1-3].
In the last years, surface electromyography (EMG) was largely utilised for clinical evaluations [4,5]: in fact, by analysing the frequency spectrum of the surface electromyographic signal, it is possible to notice a shift towards lower frequencies during a fatiguing exercise [6,7]. Until now, the tests concentrated on isometric contractions in which the electromyographic signal can be considered with some approximation as stationary.
We combined the isokinetic method to the surface electromyography [8,9] and the NIR spectroscopic technique in dynamic conditions. The modifications of striated muscle oxygenation can be measured by means of reflectance spectroscopy in the near infrared range between 700 and 1000 nm [10,11]. These variations have been extensively studied during isometric contractions, but have not yet been analysed in presence of isokinetic contractions [12,15].
In order to investigate the oxygen consumption and blood perfusion, two different wavelengths (750 and 810 nm) have been used to monitor the oxygenated and deoxygenated haemoglobin (HbO₂ and Hb) in...
the examined tissues. These particular wavelengths were chosen because of the absorption spectra of HbO₂ and Hb: at 760 nm the spectrum of Hb presents an absorption peak, whereas at 805 nm (isosbestic point) the two absorption spectra are equal.

2. MATERIALS AND METHODS

2.1 NIR spectroscopy
The NIR system developed by our group was basically composed by three parts: the instrument box, the optical probe and a lap-top computer. In particular, the optical probe coupled the subject to the instrument by ensuring the electrical insulation. The instrument utilised two laser diodes (Sharp LT010MD and LT030MD) with output frequencies centred around 750 and 810 nm respectively and one silicon photodiode (Hamamatsu 1226-8BK) with low-pass optical filter for visible light cut-off. The two laser diodes were small, reliable, easily coupled with the optical fibres and their output power was stabilised through a feedback monitoring photodiode. The silicon photodiode was chosen instead of the photomultiplier tube to improve the intrinsic safety of the instrument and to get, with a good preamplifier circuit, a more stable and noiseless detection system. The two laser diodes were turned on and off by a digital timing circuit. A single channel for backscattered light collection was used in order to minimise the noise produced by the front-end electronics. The rejection of stray-light was achieved by subtracting its contribution from the overall signal when the lasers were off.

An optical probe consisting of three glass core (50 μm diameter) fibre bundles was used to carry the excitation light to the tissue and to collect the backscattered light. The collecting fibre bundle (2 mm diameter) was placed at a distance of 12 mm from the excitation ones (1.2 mm diameter). The detected signal was inversely proportional to the tissue absorption at the working wavelength, so that a signal decrease corresponded to an increase of the absorber concentration in the examined volume.

A microcontroller inside the instrument performed the 12 bit analog to digital conversion of the two channels and sent the data through the RS232 serial port directly to a personal computer. A specific software for the analysis and storing of recorded data was developed.

Continuous laser spectroscopy didn’t yield absolute values of the oxygen saturation because of the unknown optical path length. So, the changes of tissue oxygenation were expressed in percentage units and were referred to the rest average value calculated in the baseline period at the beginning of the recording session. Moreover, 750/810 reflectance ratio was computed: this parameter provided a measure of deoxygenated haemoglobin-myoglobin, independently from the variations of total blood volume in the examined tissue. A good reproducibility in the measurement of skeletal muscle oxygenation was previously demonstrated [16,20].

2.3 Surface EMG and torque
The surface electrodes (Ag/AgCl type) were placed on four different muscles: vastus lateralis, vastus medialis, semitendinosus and biceps femoris.

The EMG signal was amplified and frequency limited with a 500 Hz low-pass filter [21]. A 12 bit analog to digital converter working at 2000 samples/sec interfaced the EMG apparatus to an IBM compatible PC with software facility for frequency spectrum analysis. In particular, for each subject the median frequency derived from the power spectral density (each spectrum was calculated over a 0.5 sec period...
and the median frequency was defined as the limit corresponding to a half of the total power) was calculated.
Muscular performance in isokinetic exercise was assessed through the use of a specialised dynamometer with accommodating resistance (CYBEX 2000 by Lumex Inc.). The muscular effort applied by the subject was met with proportional resistance applied by the dynamometer in the opposite direction. Thus the instrument maintained a constant angular velocity with axis of rotation of the dynamometer aligned to the axis of the joint to be tested (knee). A computer controlled the device and the user was allowed to set the value of angular velocity and the number of repeated flexo-extensions of the leg. A specific software processed the signal providing a set of mechanical parameters such as torque, average power, total work and endurance ratio for both the extensor and flexor muscles.

3. EXPERIMENTAL PROTOCOL

The test consisted in a series of 30 repetitions of knee flexo-extensions in isokinetic conditions (Fig. 1). A preliminary group of 8 male healthy subjects aging between 20 and 30 years was studied. All the subjects had good muscular tone and a poor adipose tissue. After one minute of baseline acquisition, needed by the spectroscopic instrument for the reference determination, 30 repetitions of knee flexo-extensions at 180 degrees/sec were performed. At the end of the exercise, 3 minutes of recovery time were recorded.

Fig. 1: Description of the experimental set-up with NIR instrument, isokinetic dynamometer and EMG apparatus.
4. RESULTS

We did not find significant correlation between the percentage decreases of median frequency and corresponding torque peak, whereas a correlation between the decreases of the median frequency of extensors and flexors seemed to be present (Fig. 2).

Fig. 2: Time course of extensors and flexors torque and median frequency from the surface EMG of vastus medialis (vm), vastus lateralis (vl), semitendinosus (st) and biceps femoris (bf) niuscles during a 30 repetitions test (180 degrees/sec).

The percentage decreases of vastus medialis and biceps femoris muscles were compared with a t-test respectively to the decreases of vastus lateralis and semitendinosus. The results indicated that the median frequency of vastus medialis and biceps femoris showed more consistent variations, probably connected to the different composition of muscle fibres.

The isokinetic exercise produced a typical pattern for both the 810 and 750/810 ratio signals. In particular, the 810 signal showed an almost rectangular step during the exercise because of the squeezing effect of muscular contraction. Moreover, a steep deoxygenation was indicated by the decrease of 750/810 ratio signal, followed by a slow recovery trend (Fig. 3a).

It is also interesting to outline how in some cases the flexo-extensions produced by the subject were clearly visible as oscillations of the 750/810 ratio signal, superimposed to a mean flat value (Fig. 3b).

Nevertheless, a certain degree of variability was noticed among the subjects, preventing any possible correlation with the EMG and the mechanical parameters.
One subject was re-tested for a total of 6 experiments and a good correlation coefficient between the total work and the recovery time $T/2_R$ ($r^2 = 0.86$, $p < 0.0001$) was obtained.

Fig. 3a: Time course of the 810 and 750/810 ratio signals during a 30 repetitions test (180 degrees/sec).

Fig. 3b: Time course of 750/810 ratio signal showing an oscillatory pattern during isokinetic contractions.
5. DISCUSSION

The goals of this work were to explore the technical feasibility of surface EMG recording during rehabilitation tests, in order to apply the same fatigue parameters largely utilised in static conditions, and to investigate the reliability of spectroscopic signal as an alternative technique in isokinetic contractions studies.

The preliminary results were encouraging and showed a systematic trend in median frequency decrease for what concerns the EMG signal and a good reproducibility for NIR spectroscopic signal. The lack of correlation between the EMG and NIR parameters was due to the hard repeatability of various external factors and also because the two techniques probably detect different aspects of muscular contraction and metabolic fatigue.

The most interesting applications seem to be connected to the rehabilitation training where the inter-subject variability is eliminated and a relationship between the two techniques and the mechanical parameters could be found.

REFERENCES