Near-infrared spectroscopy and the tilting table protocol: a novel method to study the blood flow and the oxygen consumption in tissues

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ABSTRACT

We present a novel technique based on tilting the bed where the subject is lying, to non-invasively measure the tissue blood flow (*BF*) and oxygen consumption (*OC*) with near-infrared (NIR) spectroscopy. We used a NIR, frequency domain spectrometer to measure the concentrations of oxy-hemoglobin ([HbO_2]), deoxy-hemoglobin ([Hb]) and total hemoglobin (THC) in the calf muscle of human subjects. The subject was lying horizontally, and after a baseline acquisition, the bed was tilted by 10 degrees (feet down, head up). This position was kept for 1 min, then the subject was brought back to the horizontal position. This tilting procedure caused variations in the calf [HbO_2], [Hb], and THC similar to those observed during a pneumatic-cuff-induced venous occlusion. The increasing rate of THC and [Hb] caused by tilting allowed the calculation of blood flow and oxygen consumption. We found a quantitative agreement between the values of *BF* (*OC*) measured with the tilting table and with the venous occlusion protocols. On the 26 subjects examined with the tilting table protocol, we found population average values of $BF = 1.51$ ml $(100\text{ml})^{-1}\cdot\text{min}^{-1}$ and $OC = 6.10$ $\mu\text{mol}\cdot(100\text{ml})^{-1}\cdot\text{min}^{-1}$.

Keywords: near infra-red, tissue spectroscopy, blood flow, oxygen consumption, frequency-domain.

1. INTRODUCTION

The assessment of blood flow and oxygen consumption is an issue of interest in many medical fields, such as physiology, pharmacology, endocrinology, sport and vascular medicine. There are numerous techniques that allow the non-invasive measurement of blood flow (*BF*), the most common of which are nuclear magnetic resonance (NMR),[1] laser Doppler flowmetry (LDF),[2-4] Doppler ultrasound,[5] thermal clearance[6] and plethysmography.[7,8] None of these techniques allows the measurement of blood flow directly in muscles: NMR and Doppler ultrasound only provide qualitative information on the blood flow in large to medium blood vessels,[1,8] laser Doppler flowmetry and thermal clearance techniques are limited to superficial skin flow,[3,8,9] and plethysmography assesses the global blood flow in the wide volume element investigated, typically the arm or calf muscle, without being able to discriminate among the different contributions that skin, vessels, muscles, tendons and bones give to the reading.[8,10] In the last few years, near infra-red spectroscopy (NIRS) has been used in conjunction with the venous occlusion protocol to simultaneously measure the blood flow and the oxygen consumption in muscles.[11,12] It has been proposed that the rate of increase in the concentration of total hemoglobin and deoxy-hemoglobin in the seconds that follow the occlusion allows one to calculate the blood flow and the oxygen consumption, respectively.[10] Since the measurement is carried out *in situ*, the venous occlusion in conjunction with NIRS provides information on the local *BF* and *OC*. These parameters can vary considerably both in time and in space in response to various physiological effects, such as thermoregulation, psychological state, exercise, etc. Therefore, a study of the spatial distribution of local blood flow and oxygen consumption at rest could give a better insight into some aspects of muscle physiology which are not completely understood yet. In spite of these advantages, the venous occlusion protocol presents some intrinsic limitations. First, it can only be applied to the arm or calf muscle. Second, the pressure of 40-60 mmHg currently used in the protocol is not always sufficient to bring about a complete venous occlusion.

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In this paper, we present a novel approach, the tilting table protocol in conjunction with NIRS, which is based on tilting the bed where the subject is lying at an angle of about 10 degrees to the horizontal. The variations of oxy-, deoxy-, and total hemoglobin concentration are measured in real time by a NIR, frequency domain, tissue oximeter. This protocol potentially allows the measurement of blood flow and oxygen consumption in muscles, at rest, preserving all the advantages of near infra-red spectroscopy and overtaking the limitations of the venous occlusion protocol. We carried out a set of measurements on 26 human subjects, in order to compare the values of blood flow and oxygen consumption obtained with the tilting table protocol with those measured by the venous occlusion. We also investigated whether the rest values of blood flow and oxygen consumption can be good indicators of peripheral vascular disease (PVD).

2. SUBJECTS AND METHODS

2.1. Subjects and Preliminary Tests

We carried out a series of measurements on 26 subjects (25 men, 1 woman), with average age of 61 years (range 37-84 years) and average weight of 87 kg, (range 53-122 kg). Ten of them were control subjects, while the others had different stages of peripheral vascular disease (PVD) in one or both legs. Other pathologies, such as diabetes, heart condition or high cholesterol, were not considered in the classification of the subjects. The subjects were recruited by the Danville V. A. Medical Center (Danville, IL) where the experimental work was carried out. They first underwent a routine examination at the Vascular Lab, consisting in the measurement of segmental pressure (ankle, calf and thigh pressure scaled to brachial pressure). This study was performed using a plethysmograph (IMEXLAB9000/9100). Since we were interested in characterizing the blood flow and the oxygen consumption in the calf muscle, the state of health or the grade of disease in each limb was assessed in terms of the calf-brachial index (*CBI*), namely the ratio between the calf and the arm pressure. Values of *CBI* higher then 1.1 referred to normal subjects; values between 0.9 and 1.1 referred to mild disease; values between 0.5 and 0.9 referred to moderate disease, and values lower then 0.5 referred to severe disease.

2.2. The NIR Tissue Oximeter

We used a near infra-red (NIR), dual wavelength, frequency-domain tissue oximeter (Model No.96208, ISS Inc., Champaign. IL).[13] The wavelengths were 758 and 830 nm. The oximeter had two parallel channels, thus allowing simultaneous measurements in two distinct locations, which in the present study were the right and left calf muscles. The optical probe for each channel had eight optical fibers connected to the sources, and an optical fiber connected to the detector. The distances between the detector fiber and the source fibers ranged from 2 to 3.5 cm, by increments of 0.5 cm (Fig.1). The sources were sinusoidally modulated in intensity, at a frequency of 110 MHz, and were multiplexed so that each of them was turned on for 20 ms. The acquisition time was set to 0.64 s. The instrument was calibrated before each measurement by positioning the optical probes on a strongly scattering material of known optical properties, in order to compensate for the differences in the emission of each source.

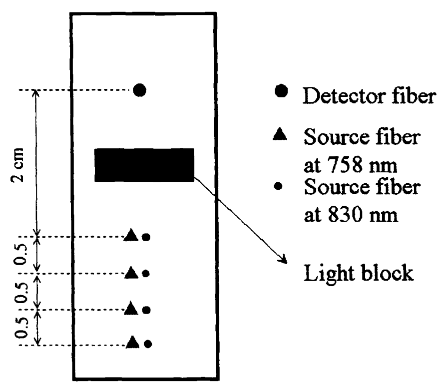


Fig.1- Geometrical arrangement of the optical fibers in the probe positioned on the calf muscle.

By measuring the distance dependence of the continuous component (DC), the alternating component (AC) and the phase of the back scattered light, the instrument measured the absorption coefficient μ_a and the reduced scattering coefficient μ_s' of the underlying tissue. From the optical absorption of the tissue at two wavelengths, the oxy-hemoglobin, deoxy-hemoglobin, total-hemoglobin concentrations and the hemoglobin saturation were calculated.[14] The time evolution of these parameters was observed during the measurement, in real time, on a computer screen.

2.3. The NIR Protocol

We performed measurements of blood flow (*BF*) and oxygen consumption (*OC*) at rest, both with the venous occlusion and the tilting table protocols, in order to compare the results obtained with the two NIR methods. Before undergoing the NIR protocols, all volunteers signed an informed consent form. The subject was lying down on a bed. The positioning of the optical probes on the calf muscles required special care. In fact, our theoretical model assumes the macroscopic homogeneity of the investigated tissue. This assumption is violated in the presence of inhomogeneities such as large blood vessels, so that we avoided placing the optical probe nearby visible superficial blood vessels.

The details of the venous occlusion protocol are reported elsewhere.[15] Briefly, two pneumatic cuffs were positioned on the right and left thighs of the subject. After a baseline acquisition, the cuffs were simultaneously inflated to a pressure of 60 mmHg, which was kept for 1 min.

Immediately after the venous occlusion protocol, we performed the tilting table protocol. The optical probes were not moved, so that the measurements obtained with the two NIR methods referred to the same position, and were therefore directly comparable. After the acquisition of a baseline, the bed was tilted, by means of an electric control, at an angle of 10 degrees to the horizontal, with the legs down and the head up (Fig. 2). The tilting procedure occurred in about 3 s. This position was kept for 1 min, and then the bed was brought back to the horizontal position in 3 s, and held still for another minute. The whole procedure was repeated three times to check for reproducibility.

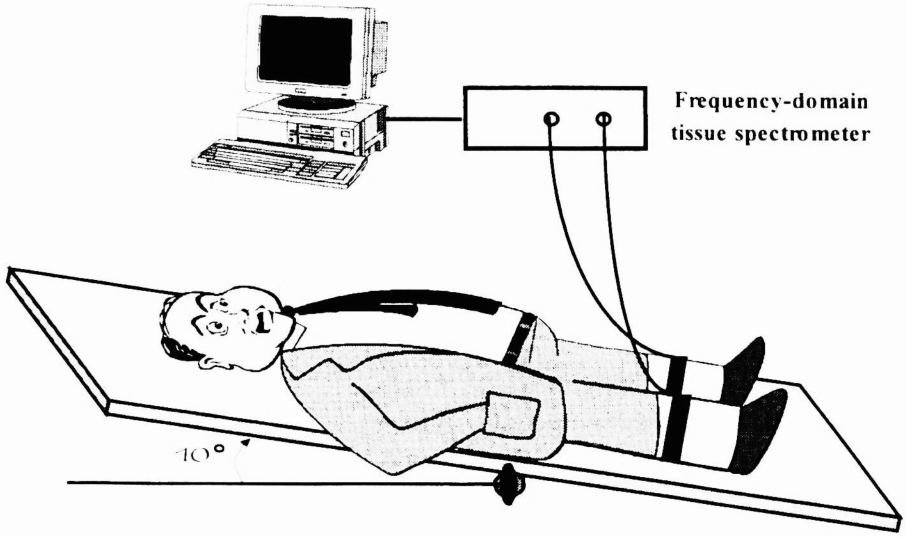


Fig.2- Schematization of the tilting table protocol. The bed is tilted by 10 degrees for one minute, while the optical probes are positioned on the calf muscle of both legs.

2.4. The Calculation of Blood Flow and Oxygen Consumption

2.4.1. Venous occlusion

It has been proposed that the rate of increase of total hemoglobin concentration (THC) and of deoxy-hemoglobin concentration ([Hb]) during venous occlusion allows the calculation of the blood flow and oxygen consumption.[10] In fact, when the venous occlusion takes place, the inflow of oxygenated arterial blood in the muscle is not altered, while the outflow of deoxygenated venous blood is inhibited. The rate of increase of total hemoglobin is therefore exclusively due to the arterial blood flow, while the increase in the deoxy-hemoglobin concentration is mostly due to a conversion of oxy-hemoglobin

[HbO2] into deoxy-hemoglobin caused by the tissue oxygen consumption, plus a small contribution from the deoxy-hemoglobin in arterial blood. Explicitly:

$$BF = \frac{1}{C}\frac{\mathrm{d(THC)}}{\mathrm{d}t}, \tag{1}$$

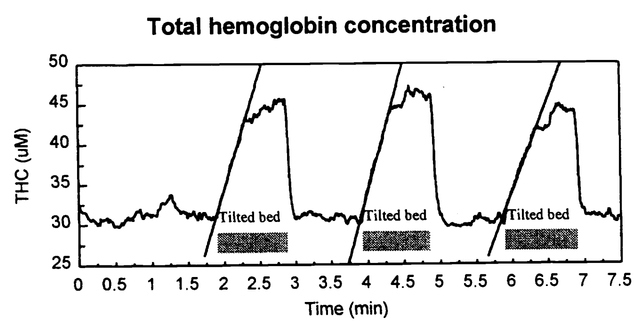
$$OC = 4\frac{\mathrm{d}}{\mathrm{d}t}\left[[\mathrm{Hb}] - \frac{100 - \mathrm{SaO_2}}{100}(\mathrm{THC})\right], \tag{2}$$

where C is the concentration of hemoglobin in the blood, and $\mathrm{SaO_2}$ is the arterial saturation in percent.

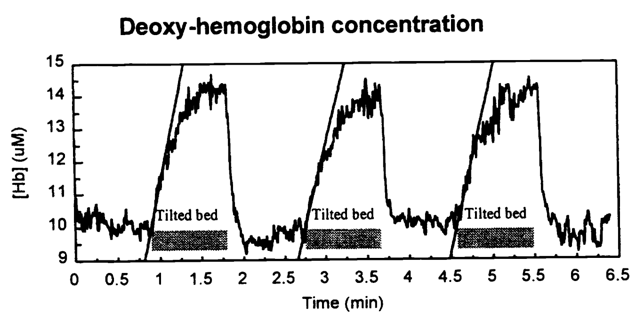
2.4.2. Tilting table

When the bed is tilted from the horizontal position by 10 degrees, lowering the legs of the patient, an increase of the total blood volume in the muscle is observed, due to the gravitational pull exerted on the blood. This is reflected by an increase in the [HbO2], [Hb] and THC (Fig.3). The venous reflux of deoxygenated blood from the limb towards the heart finds a hindrance. Starting from the qualitative agreement between the behavior of the concentrations during the venous occlusion and the tilting table protocols, we made the assumption that, although the venous flux is not completely interrupted during the tilting table protocol, in the seconds that follow the inclination of the bed, the effect on the venous circulation is analogous to that observed during the venous occlusion.

We calculated the blood flow and the oxygen consumption by performing a linear regression of the traces of THC and [Hb], from the instant in which the bed was tilted until the linear behavior is observed (Fig.3). The slopes obtained in this way were entered as the rate of change of THC and [Hb] in Eqs. (1) and (2) for *BF* and *OC*.



BF=1.23±0.36 ml $(100\mathrm{ml})^{-1}\cdot\mathrm{min}^{-1}$



OC=3.68±0.77 μmol·$(100\mathrm{ml})^{-1}\cdot\mathrm{min}^{-1}$

Fig.3-Example of the time evolution of the concentrations of total hemoglobin (THC) and of the deoxy-hemoglobin ([Hb]) during the tilting table protocol. The gray bars indicate the time during which the bed was tilted. The lines represent the data linear regression . Above each figure we reported the calculated values of blood flow and oxygen consumption and their error.

The calculation of the error which affects the blood flow and the oxygen consumption required careful consideration. There are three main contributions to the error in the hematic parameters: the first is due to the instrumental noise, the second is related to the choice of the time interval for the linear regression, the third is due to the slope variability in the three subsequent measurements on the same position. The instrumental noise was mostly due to shot noise, which is strongly dependent on the subject. To evaluate its effect on the blood flow and oxygen consumption, we considered the standard deviation error of the slope given by the linear regression, and we propagated it into the formulas for blood flow and oxygen consumption (Eqs. (1) and (2)). To quantify the error due to the choice of the time interval for the linear regression, we identified the minimum and maximum slope within the range of acceptable time intervals. The semi-difference in the values of *BF* and *OC* corresponding to these extreme slopes was taken as an estimate of the error. Generally, the three values of blood flow and oxygen consumption generated by each of the three successive measurements were found in agreement within errors. In the few cases in which they did not agree, we took the average of the three, and expressed the error with the standard deviation of the three values

3. RESULTS

3.1. Blood Flow

We were able to measure the blood flow on 36 of the 52 legs on which the measurement was carried out. The values measured with the tilting table protocol are reported in Fig.4(a). The average values of blood flow were found to be higher in legs affected by PVD than in healthy legs, but taking into account the variability among subjects, represented by the standard deviation on the values measured, the intervals broadly overlap.

3.2. Oxygen consumption

We could measure the oxygen consumption on 15 of the 52 legs on which the measurement was carried out. The results are shown in Fig.4(b).The average oxygen consumption resulted higher in legs affected by peripheral vascular disease than in healthy legs, but the values broadly overlap within variability.

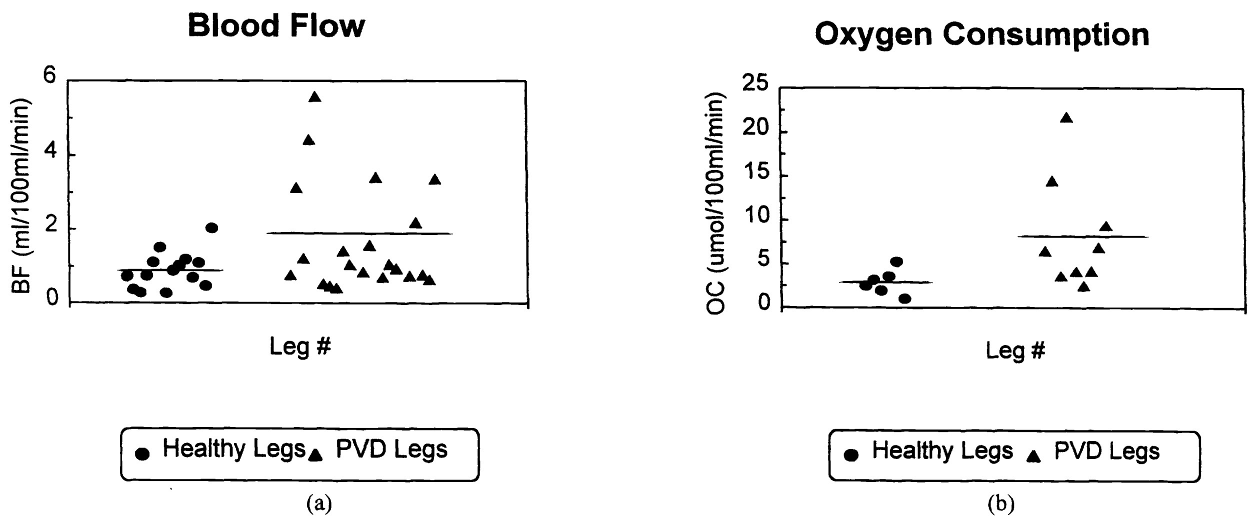


Fig.4- Summary of the values of blood flow (a) and oxygen consumption (b) measured on the subjects.

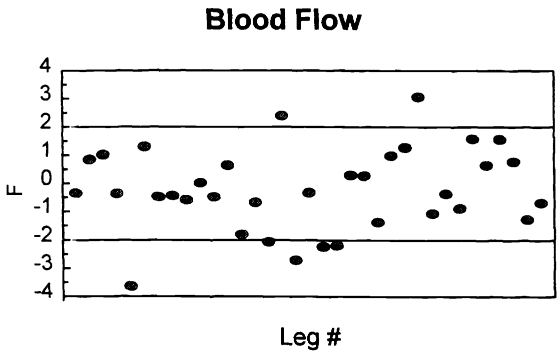
3.3. Comparison Between the Tilting Table and Venous Occlusion Protocols

We also compared the single values of blood flow and oxygen consumption measured in each subject, in each limb, with those measured in the same position with the venous occlusion protocol. For each leg we calculated the difference in the *BF (OC)* values measured with the two protocols over the sum of the errors on the two measurements:

$$F = \frac{BF_{Ven} - BF_{Tilt}}{\Delta BF_{Ven} + \Delta BF_{Tilt}} \tag{3}$$

$$G = \frac{OC_{Ven} - OC_{Tilt}}{\Delta OC_{Ven} + \Delta OC_{Tilt}} \tag{4}$$

The results are plotted in Fig.5. Practically all data are in quantitative agreement within two standard deviations.



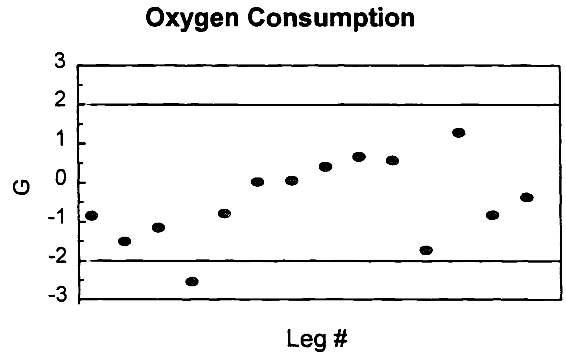
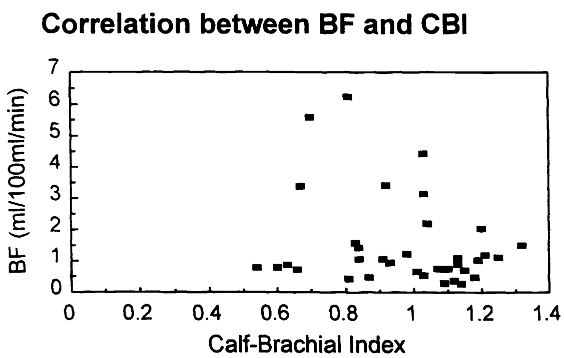


Fig.5- Summary of the agreement between the values of BF (OC) measured with the tilting table and those measured on the same position with the venous occlusion protocol. On the Y axis are reported the values of functions F and G defined by Eqs. (3) and (4).

3.4. Correlation Between Blood Flow, Oxygen Consumption, and Calf-Brachial Index

Finally, we studied the correlation between the values of blood flow and oxygen consumption measured with the near infra-red protocol and the calf-brachial indexes provided by the segmental pressure analysis. To do this, the values of blood flow and oxygen consumption of all subjects were plotted as a function of their calf-brachial indexes (Fig.6). As shown in Fig.6, no correlation was found.



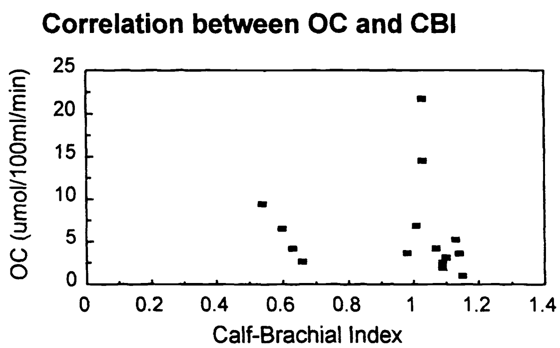


Fig.6- Values of blood flow (*BF*) and oxygen consumption (*OC*) measured on all subjects as a function of the Calf-Brachial Index values.

4. DISCUSSION

4.1. Selection of the Data

We could not calculate the blood flow and the oxygen consumption in all the cases considered. We had to discard those cases in which, although the increases in the concentrations of total hemoglobin and deoxy-hemoglobin were recognizable, they were too low to allow the calculation of the slopes. We also discarded those cases in which the concentrations showed an abnormal time behavior, or did not appreciably increase during the tilting table protocol. Finally, we discarded the calculated values of blood flow and oxygen consumption which showed an error higher than 40%. For the values presented, the average error for the blood flow is 22%, while for the oxygen consumption is 25%.

4.1.1. Low signal to noise ratio

One of the reasons that some data could not be processed was the low value of the signal to noise ratio: in some cases the increase in the concentrations of total hemoglobin and deoxy-hemoglobin during the tilting table protocol were comparable to the instrumental noise, thus not allowing the calculation of the slopes. This problem affected the deoxy-hemoglobin, and therefore the oxygen consumption determination, to a larger extent then the total hemoglobin. The reason is that the concentration increase observed in the deoxy- is generally smaller than that for the total hemoglobin. In fact, only 15 of the 52 legs measured lead to the evaluation of the oxygen consumption with an error lower than 40%. A reduction of the instrumental noise could obviously reduce the problem encountered, but we point out that in the majority of the cases, the instrumental noise was not a limiting factor in the calculation of the slopes. Another relevant parameter could be the position where the probe was located for the measurement; it cannot be excluded that the low increase in the concentrations observed in some cases could be a result of the vascular structure of the underlying tissue. It is therefore possible that moving the optical probe to a different location could lead to a trace showing larger dynamics.

4.1.2. Abnormal traces

In some cases the reason why the slopes could not be calculated was the time evolution of the concentrations of total hemoglobin and deoxy-hemoglobin during the tilting table protocol. In fact, in some cases the tilting table did not bring about any significant change in the concentrations. In some other cases, the time variation of the concentrations was not the one expected, most probably as a result of some stabilization processes. It is possible that in these cases the probe was not located on an ideal position. It is also possible that a tilting angle of 10 degrees is not sufficient to bring about an observable effect in all subjects. In this respect, increasing the tilting angle to 15 or 20 degrees could help in overcoming this limitation.

4.2. Blood Flow and Oxygen Consumption

The average values for the blood flow and oxygen consumption measured on the subjects examined by us are: $BF = 1.51$ ml $(100\text{ml})^{-1}\cdot\text{min}^{-1}$ and $OC = 6.10$ μmol$\cdot(100\text{ml})^{-1}\cdot\text{min}^{-1}$. These values are in agreement with those measured with NIRS and reported in literature.[10,11,15,16]

As it can be seen in Fig.5, almost all values of blood flow and oxygen consumption measured with the tilting table protocol and the venous occlusion protocol[17] are in quantitative agreement within two standard deviations. This confirms that the tilting table protocol provides an alternative to the venous occlusion protocol in the measurement of local blood flow and oxygen consumption in muscles with NIR spectroscopy.

We found no correlation between the values of blood flow and oxygen consumption, and the calf-brachial pressure index. One could have expected that in the presence of an arterial occlusion due to peripheral vascular disease the blood flow should be lower, proving the insufficiency in the arterial delivery. This was not found, and the measurement of normal values of blood flow and oxygen consumption in PVD patients at rest is explained in terms of the collateral circulation, which is developed soon after an occlusion in the arteries takes place, and which bypasses the occlusion by consistently developing the capillary net in the surroundings. Significant differences in the values of blood flow and oxygen consumption between PVD patients and control subjects can be evidenced with NIRS during exercise, where the oxygen demand in the muscle is suddenly and conspicuously increased due to physical activity.[10]

5. CONCLUSIONS

This study proved that the time variations in the concentrations of total and deoxy- hemoglobin, measured with near infra-red spectroscopy during the tilting table protocol, allow the calculation of local blood flow and oxygen in muscles. We found no correlation between the rest values of blood flow and oxygen consumption and the presence of peripheral vascular disease.

In conclusion, we have introduced the tilting table protocol as a promising technique for the non-invasive measurement of blood flow and oxygen consumption in muscles. This novel protocol involves no discomfort and a minimal perturbation of the hemodynamics of the subject. Among its possible applications, we envision the measurement of cerebral blood flow and oxygen consumption.

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