Calf muscle blood flow and oxygen consumption measured with near-infrared spectroscopy during venous occlusion

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ABSTRACT

We present non-invasive measurements of the calf muscle blood flow (BF) and oxygen consumption (OC) by near-infrared spectroscopy. We used a frequency domain tissue oximeter (modulation frequency: 110 MHz, wavelengths: 758 and 830 nm) to measure in real time (acquisition time: 0.64 s) the hemoglobin concentration and saturation. After 1-min of baseline acquisition, we achieved venous occlusion by inflating a pneumatic cuff on the subject's thigh to a pressure of 60 mmHg. The cuff was released after 1 min. The baseline/inflation/release procedure was repeated 3 times to verify reproducibility. We calculated the BF and OC from the initial rate of increase of the total hemoglobin and deoxy-hemoglobin concentration immediately after the onset of venous occlusion. We examined 8 healthy subjects and 18 patients affected by peripheral vascular disease (PVD) in 1 or 2 legs to investigate whether muscle BF and OC at rest can be useful indicators of vascular insufficiency. In healthy legs, we obtained average values of BF=0.73 ml/(100ml)/min and OC=0.10 ml/(100g)/min. The corresponding average values found in legs affected by PVD are BF=1.39 ml/(100ml)/min and OC=0.16 ml/(100g)/min. The ranges of values of BF and OC measured in the healthy legs broadly overlap with the corresponding ranges measured in the PVD legs.

Keywords: near infrared spectroscopy, blood flow, oxygen consumption, peripheral vascular disease.

1. INTRODUCTION

1.1. Peripheral Vascular Disease (PVD)

Peripheral vascular disease (PVD) is caused by a narrowing of the blood vessels that carry blood to leg and arm muscles. There are two types of disorders[1]: *(1) Functional peripheral vascular diseases* do not involve defects in the structure of the blood vessels. They are short-term effects, may be reversed (example: Raynaud's disease) and can be triggered by cold temperatures, emotional stress or smoking. *(2) Organic peripheral vascular diseases* are caused by structural changes in the blood vessels (such as inflammation and tissue damage). An example is Buerger's disease, a chronic inflammatory disease found chiefly in the peripheral arteries and veins of the extremities. This disease commonly happens to men who smoke cigarettes. Symptoms include rest pain in the legs or feet, clammy and cold skin, and a diminished sense of heat and cold[1].

Despite the fact that this disease is very common, the effects of ischaemic atherosclerotic occlusive disease on the peripheral oxygen delivery (blood flow) and muscle oxygen consumption are not well characterized. Thus, the determination of blood flow and oxygen consumption in skeletal muscle in the presence of arterial diseases is of interest.

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1.2. Different Techniques for Blood Flow Measurements

There are few methods that are able to assess blood flow of the skeletal muscle, but not many to assess oxygen consumption. There are three main categories of methods and some of them will be shortly described[2-5]:

- *anatomical* (angiography, pathology, nuclear magnetic resonance imaging)
- *direct* (plethysmography, Xenon 133 inhalation, laser Doppler flowmetry, Doppler ultrasound)
- *indirect* (ankle-arm blood pressure, nuclear magnetic resonance spectroscopy, near infrared spectroscopy)

Plethysmography measures regional limb blood flow, at rest, using a cuff, inflated at 40-60 mmHg, which induces venous occlusion. The increase in the volume of the limb, recorded in time, allows blood flow determination. All types of plethysmography methods have 85% accuracy and 6% - 20% overall variability among patients.[2] The rest blood flow values, for a normal subject are about 3.3±0.7 ml/100ml tissue/min.[2] Although this technique is widely used, it has some limitations: the fractional limb blood flow to skin and subcutaneous tissue is minimal at rest and increased during pregnancy, during and after exercise and in response to high temperatures. Thus, plethysmography can be performed only at rest and in certain conditions. Motion artifacts also limits this technique. This technique can not be used in subjects with diabetes[3] because of the phlebophaty, which does not allow venous occlusion protocol application.

Xenon 133 inhalation is one of the clearance techniques that measures the blood flow of an organ by the clearance of a tracer, radioactive (xenon 133[2] and technetium[4]) or dye introduced via arterial, venous or intramuscular injection. The blood flow is determined by the rate of the washout of the tracer. This technique has 25% variability.[2] The rest blood flow values, for normal subjects, are 10 - 20 ml/100ml/min, and for subjects with arterial occlusive disease 2-8 ml/100ml/min.[4] The clearance techniques have a clear disadvantage of being invasive.

Nuclear magnetic resonance imaging can be used in two different approaches: the time-of-flight (measures the proton partial saturation effects caused by flow perpendicular to the image plane), or Fourier methods (uses phase-shift to solve protons according to their velocity).[3,6] The problems of this technique are the high signal loss encountered when velocity of flow increases and difficulties in quantifying blood flow, which is oblique to the axis of the magnet.[2] Also, this method has a high cost and can not be performed on subjects with metal implants.[3]

Laser Doppler flowmetry, optically measures the flow in small regional volumes, primarily on superficial tissues. Using Doppler principle, this method detects the frequency shift of the laser light imparted by moving red blood cells. The blood flow is linearly related to the mean frequency shift of the scattered light. This technique has the advantage of a small probe, high spatial resolution and rapid response, but there are also some drawbacks, as the usage of calibration factors and the extreme sensitivity to any motion and expensive equipment requirement.[2] This technique also showed variability among patients of a factor of five and differences from site to site on a given subject.[7]

As it was shown, all these methods have disadvantages. Some are invasive (angiography, pathology, Xenon 133 inhalation). Some determine the general condition of the muscle, being non-specific techniques (plethysmography, ankle-arm blood pressure). Some assess superficial blood flow (laser Doppler flowmetry), or have high sensitivity to movements (magnetic resonance imaging). Recently, a new noninvasive method, near infrared spectroscopy has offered a valuable alternative to diagnose and monitor peripheral vascular disease. In the near infrared spectral region the absorption properties of biological tissues are primarily due to the oxygenation level of the tissue hemoglobin. Furthermore, in the near infrared region (700 - 900 nm) light penetrates several centimeters into tissue thus, allowing deep tissue measurements.

Near infrared spectroscopy techniques (NIR) measure blood flow and oxygen consumption of the muscle by illumination of the intact tissue with selective wavelengths[3,7]. In order to determine blood flow and oxygen consumption on skeletal muscle, it has been shown that some type of perturbation is necessary. These perturbations had previously been venous or arterial occlusion. Recently, with the new techniques, there are also other possibilities. One can use a breathing protocol which involves different content of oxygen[18] or other gases (CO_2, N_2 or a combination)[8,9] and a new protocol, namely the tilting table protocol.[10] There are important advantages of the new non-invasive methods: low cost of the instrumentation, portable system, possibility of a continuous measurement, can be performed at rest as well as during exercise, can probe different tissues (muscle, brain). This technique has a big potential for skeletal muscle characterization in ill and elderly people, as it was shown in previous studies.[2,5,11]

In our present study, we used near infrared frequency-domain technique in conjunction with venous occlusion to determine the blood flow and oxygen consumption on the calf muscle of healthy subjects and subjects with peripheral vascular disease, at rest.

2. EXPERIMENTAL AND THEORETICAL METHOD

2.1. Near Infrared Frequency-Domain Technique

Near infrared (NIR) frequency-domain spectroscopy permits one to determine the absorption and scattering coefficients of a strongly scattering medium such as tissue. Previous studies were testing the near infrared tissue spectroscopy method for determination of the BF and OC in patients with PVD and some indications showed that the method is promising.[11,12]

Near infrared frequency-domain techniques are based on sinusoidally modulated intensity of the light source and on a phase detection system to measure the amplitude of the intensity oscillations (AC component), the average intensity (DC component) and the phase (Φ) of the detected light.[13,14] Measuring these quantities (AC, DC and Φ) at different source-detector separations, the optical coefficients (absorption, μ_a, and reduced scattering, μ_s') are determined (for a semi-infinite geometry using either DC and phase or AC and phase)[12]. We used a near infrared frequency-domain oximeter (Model 96208, ISS, Inc., Champaign, IL) with modulation frequency of 110 MHz, operating at two wavelengths (4 laser diodes at 758 nm and 4 laser diodes at 830 nm). We used 2 channels in order to measure simultaneously both calf muscles (left and right leg) of each subject. The measurements were performed in real time (acquisition time: 0.64 s). The instrument is shown in Fig. 1.

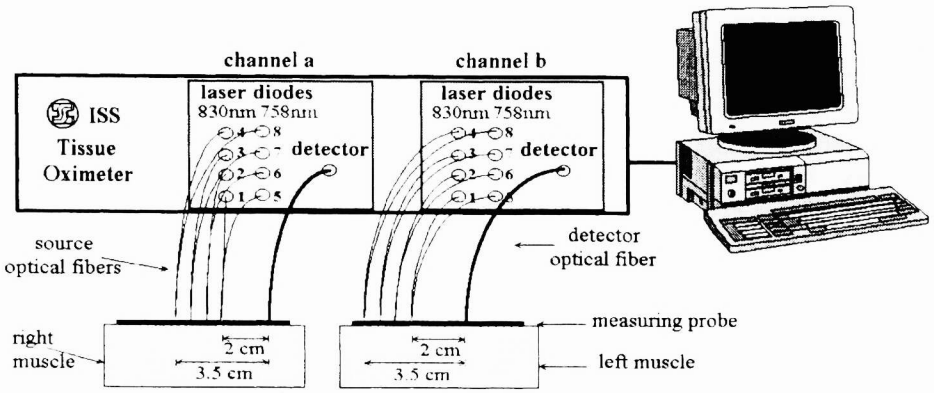


Fig.1. Experimental apparatus - near infrared tissue oximeter.

The equations for μ_a and μ_s' have been previously derived from the slopes (S) of the functions: $f(r, DC, \mu_a, \mu_s')$ versus r and $h(r, \Phi, \mu_a, \mu_s')$ versus r.[14] Using an iterative process, the unknown optical coefficients are found. The two wavelengths (758 nm, 830 nm) are necessary to determine the deoxyhemoglobin and the oxyhemoglobin concentrations in tissues.[15]

From the oxy- and deoxy-hemoglobin concentrations we can determine the blood flow and oxygen consumption of the skeletal muscle. In order to determine the BF and OC in the skeletal muscle of a subject, at rest, we calculated the initial rate of increase in the total (THC) and deoxy-hemoglobin ([Hb]) concentrations immediately after the onset of a venous occlusion.[16,17] The equations for BF and OC are:

$$BF = \frac{1}{C}\frac{d[THC]}{dt} \tag{1}$$

$$CO = 4\frac{d}{dt}\left[[Hb] - \frac{100 - SaO_2}{100}[THC]\right] \tag{2}$$

where C is hemoglobin concentration in the blood and SaO_2 is the arterial saturation in percent.

2.2. Experimental Procedures

2.2.1. Subjects

The subjects examined in this study were provided by the Danville VA Medical Center. The protocol was also approved by Danville VA Medical Center. All subjects were informed about the procedure and gave their written consent. The optical measurements were performed on 26 subjects (25 male, 1 female), 8 controls and 18 patients with different stages of peripheral vascular disease (PVD) on one or both legs. The physical characteristics of the subjects were: average age 61.5 years (range 37 - 84 years), average height 174 cm (range 160 - 185 cm) and average weight 87 kg (range 53 - 121 kg). Adipose tissue thickness in the region of detection was 3-18 mm. One subject was non-smoker, 14 were smokers and 11 were smokers who quit smoking for sometime. Only 1 subjects had no other physical conditions. Most of the patients had one or more of the following conditions: heart disease, diabetes, PVD, hypertension, angina, stroke or surgery. Thus, all subjects but one were under some type of medication (as vasodilator drugs, anticoagulant drugs).

2.2.2. Protocol and tests

The subject was laid in a supine position on a comfortable clinical bed. A pneumatic cuff was placed on the each of the right and left thigh and was used to apply venous occlusion at 60 mmHg by manual inflation. The inflation time was about 6 seconds. The optical probes were placed on each calf (right and left), on the inner part (*gastrocnimius* muscle), avoiding the main veins and arteries. The subject's legs were elevated by about 10 cm to avoid the contact of the optical probes with the bed. After 1-min of baseline acquisition, we achieved venous occlusion. After 1-min the inflation cuff was released. The baseline/inflation/release procedure was repeated 3 times to verify for reproducibility. The data acquisition was acquired automatically on the computer. For a good indication of the inflation onset, we marked these events during the data acquisition.

Prior to our study, a maximum venous outflow (MVO) test was performed using a pneumoplethysmography (IMEXLAB 9000/9100), in order to evaluate the pressures in the subject's arm and legs. The ratio of these pressures is the calf brachial index (CBI), an indicator of the stage of the arterial disease in the calf muscle. For CBI > 1.1, the subject circulation is normal, for CBI = 0.9 – 1.1 mildly abnormal, for CBI = 0.5 - 0.9 moderately abnormal, and for CBI < 0.5 severely abnormal. This test is used by Danville VA Medical Center for an estimation of the disease.

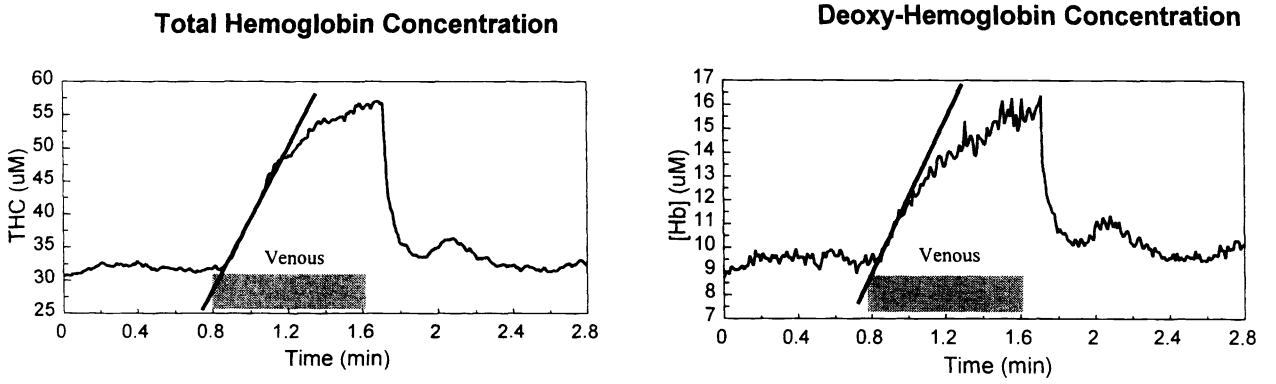
2.2.3. Blood flow and oxygen consumption measurements

It has been proposed that the rates of increase of total hemoglobin concentration (THC) and of deoxy-hemoglobin concentration ([Hb]) immediately after the onset of a venous occlusion allow the calculation of the blood flow and oxygen consumption.[16,17] When we apply a venous occlusion, the inflow of arterial blood (oxygenated) is not affected, while the venous 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 registered in the deoxy-hemoglobin is due to the conversion of oxy-hemoglobin [HbO2] into deoxy-hemoglobin, determined by the oxygen uptake for the muscle metabolism.

We calculated the BF (OC) using a linear regression of the of THC ([Hb]) traces in the initial seconds after the onset of the venous occlusion. The slope of the line obtained was substituted in the expressions for BF (Eq. (1)) and OC (Eq. (2)). For error evaluation, we considered three main contributions. One due to the measurement noise effecting the determination of the THC and [Hb], other related to the uncertainty in the time interval considered in the regression, and another from the variability in the BF and OC values calculated from the three waveforms. The first error was considered to be the standard deviation of the slope of the linear regression. For the second one, we considered two linear regressions possible in the time interval considered and the error was the semi-difference in the BF and OC corespondent. We calculated the BF and OC for each of the three waveforms, for each subject, and we obtained a weighted average of the three values. For the cases when the three waveforms gave different results we considered the error as the standard deviation of the three values which was considered the third source of errors.

3. RESULTS AND DISCUSSION

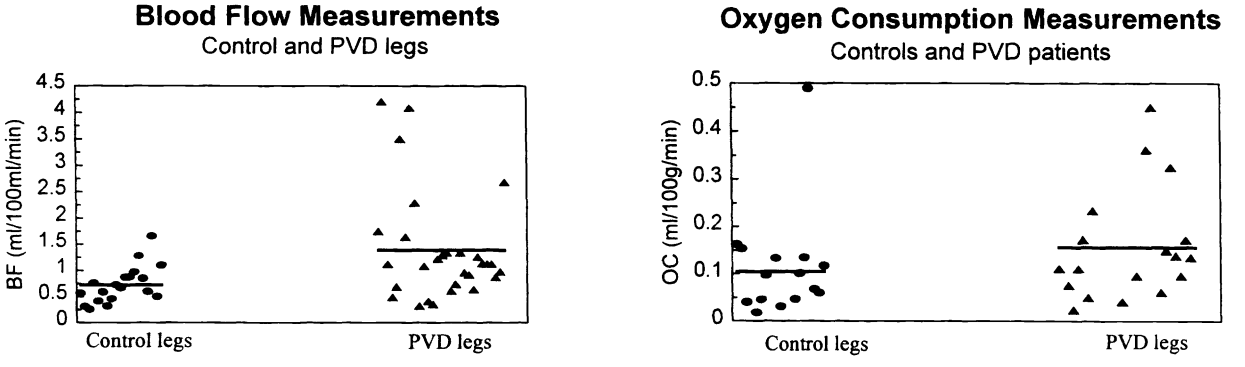
First, we determined the BF and OC for all of the 26 subjects. An example of the time traces of THC and [Hb] during the venous occlusion protocol, is presented in Fig. 2.



(a) BF = 2.19 ± 0.04 (ml/100ml/min) (b) OC = 0.12 ± 0.02 (ml/100g/min)

Fig.2. Total hemoglobin concentration (a), and deoxy-hemoglobin concentration (b) versus time. The lines represent the results of the regression. The slopes of these lines are used to calculate the BF and OC.

The BF and OC values and the variability among the subjects found with the near infrared frequency-domain oximeter using venous occlusion protocol, are presented in Fig. 3.



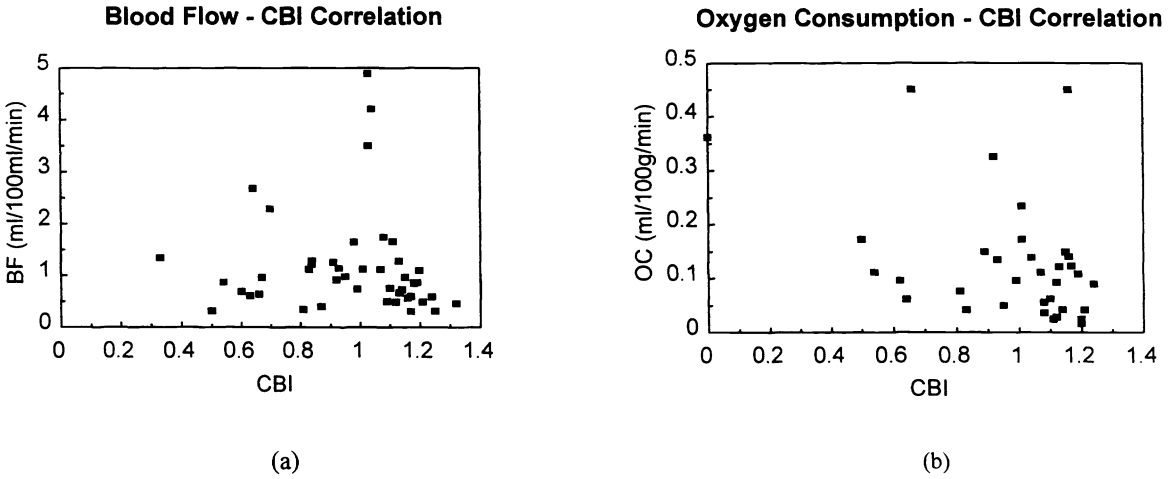
(a) (b)

Fig.3. Blood flow (BF) and oxygen consumption (OC) values with the averages for control and PVD legs using venous occlusion protocol.

The BF and OC values are in agreement with those reported in the literature for the venous occlusion.[16-19] As shown in Fig. 3, the BF and OC in the muscle are not able to discriminate between a control subject and one affected by PVD due to large variability among subjects. One explanation of this fact is that all our subjects were under medication (vasodilator drugs, anticoagulant drugs). It was shown that, for example, vasodilator drugs can increase the resting blood flow in limb[1]. Thus, this might be an important factor in the large variability of our results.

Second, we studied the correlation between the values of BF (OC) measured, at rest, with this optical method and the CBI obtained with the plethysmography exam.

From the maximum venous outflow (MVO) test, we obtained the CBI for each of the calves of all the subjects. We plotted the blood flow and the oxygen consumption values as a function of the CBI, as shown in Fig. 4.



(a) (b)

Fig.4. Correlation between calf-brachial index (CBI) and blood flow (BF) (a) and oxygen consumption (OC) (b) using venous occlusion protocol.

No correlation was found between different conditions of PVD, given by the CBI, and the parameters measured by the near infrared method. This might be explained as an effect of the collateral circulation, which is developed soon after the occurrence of an occlusion of the arteries or as effect of the medication, as mentioned before.

We also divided the BF and OC for the controls and patients in different categories to check for any correlation between the values and different physical conditions of the subjects (as age groups, disease groups, smokers and non-smokers). We found no correlation between BF (OC) and different conditions.

4. CONCLUSION

Although we found a good reproducibility of the BF and OC values over the three venous occlusions performed each time on each subject, no difference was found between the subjects with different degrees of PVD and controls, due to large variability among the subjects. The same conclusions are also reported in reference 19. Also, no correlation was shown between our results and CBI, which was not surprising since the NIR method measures local parameters while CBI measures an overall stage of the tissue. No correlation with age, weight or disease was found.

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