A Dynamic Objective Evaluation of Peripheral Arterial Disease by Near-Infrared Spectroscopy

F. Manfredini a,b,e,* A.M. Malagoni a, M. Felisatti a, S. Mandini a, F. Mascoli a,c, R. Manfredini a,d, N. Basaglia e, P. Zamboni a

Vascular Diseases Center, University of Ferrara, Ferrara, Italy
Center for Biomedical Studies applied to Sport, University of Ferrara, Italy
Vascular and Endovascular Surgery Unit, Department of Surgery, University of Ferrara, Ferrara, Italy
Clinica Medica, Department of Clinical and Experimental Medicine, University of Ferrara, Ferrara, Italy
Department of Rehabilitation Medicine, S. Anna Hospital, Ferrara, Italy

Submitted 9 March 2009; accepted 6 June 2009
Available online 21 July 2009

KEYWORDS
Near-infrared spectroscopy; Peripheral vascular disease; Intermittent claudication; Exercise tests

Abstract Objectives: Near-Infrared Spectroscopy (NIRS), suitable for dynamic measurements, is not routinely used for peripheral arterial disease (PAD). We propose a dynamic NIRS-based measurement to quantify variations in muscle metabolism in PAD.

Method: Sixty-seven consecutive PAD patients (males = 56, age 71.6 ± 8.7 years) and 28 healthy subjects (males = 12, age 30.4 ± 11.9 years) were studied. An echo-colour Doppler (ECD) was performed and the ankle–brachial index (ABI) was calculated. Participants performed an incremental treadmill test with NIRS probes on the gastrocnemius. Variations in oxygenated (HbO2), deoxygenated (HHb), total (tHb = HbO2 + HHb), and differential (dHb = HbO2 – HHb) haemoglobin were recorded and quantified as area-under-curve (AUC) within the range 1.7–3.0 km h⁻¹. Heart rate was recorded, and the number of beats in the same interval was calculated (dHr).

Results: O₂HbAUC, HHbAUC and dHbAUC differed between diseased and non-diseased legs (P < 0.0001) and exhibited different patterns related to PAD severity according to the ABI value. A compensatory heart rate increase was observed in PAD patients. Compared with the ECD positivity for occlusions/stenoses or multiple plaques, only the receiver-operating characteristic (ROC) analysis of dHbAUC (area = 0.932, P < 0.0001) showed a sensitivity/specificity of 87.6/93.4 for values ≤ -197 (LR + LR –: 13.36/0.13).

Conclusion: The dynamic NIRS-based test, quantifying muscle metabolic response according to presence and degree of PAD, allows the evaluation of patients with walking disabilities.

© 2009 European Society for Vascular Surgery. Published by Elsevier Ltd. All rights reserved.
Introduction

Peripheral arterial disease (PAD) affecting blood flow in the lower limbs is responsible for altered oxygen delivery to tissues and muscles during walking. Available methods or techniques to assess the presence or severity of PAD are performed mainly in static conditions. Otherwise, dynamic evaluations, such as functional tests, are related to patients’ reported symptoms.

Near-infrared spectroscopy (NIRS) allows an objective study of muscle metabolism at rest and under dynamic and post-exercise conditions,1–6 revealing different patterns in early O₂ desaturation or low muscle O₂ extraction in low-performing populations.7–12 For PAD patients, whose performance depends on both oxygen availability and its use, NIRS measurements have been proposed.13–18 However, despite the potential usefulness, its routine use remains limited for PAD assessment.19 We hypothesised that a dynamic assessment of muscle metabolism (perfusion and O₂ use) and cardiovascular response during exercise might be useful for the evaluation of patients with claudication or exertional leg pain in order to quantify the degree of metabolic disease and to determine the presence of PAD.

The aim of this study was to determine whether calf deoxygenation detected by NIRS during a progressive level test was quantifiable in a predetermined range of walking speed, whether the score would differ in legs with PAD according to the severity of the disease, and whether the presence of PAD might be recognised among the legs under study.

Subjects and Methods

Participant selection: Seventy-six consecutive patients (63 males, 13 females, age 72.1 ± 7.6 years) affected by Fontaine’s second-stage PAD with stable claudication or walking impairment were recruited for the study. Among people attending our rehabilitation laboratory (students n = 18, personnel n = 5 and relatives n = 5), 28 healthy volunteers (12 male, 16 female; age 30.4 ± 11.9) were randomly approached.

All participants provided written informed consent, and the protocol of the study was approved by the local Ethical Committee.

PAD was previously independently diagnosed in patients in the Vascular Surgery Department by means of clinical and instrumental examinations. Diagnosis was performed on the basis of a positive echo-colour Doppler (ECD) examination (Philips iU22 Ultrasound System). The abdominal aorta; common and external iliac; common, superficial and deep femoral arteries; popliteal arteries and both tibial axes were evaluated. High-resolution B-Mode imaging, complemented by pulsed wave Doppler analysis for detection of arterial obstruction, as well as peak-flow velocity for definition of critical stenosis were used according to published standards.20 ECD examinations were carried out by four expert vascular operators with comparable experience. They were blinded to all other measurements.

Fontaine second-stage PAD patients were then referred to our rehabilitation service. The written detailed reports of the ECD examinations were also forwarded.

Participant enrolment: PAD patients were excluded in the presence of contraindication or limitation to exercise (e.g., unstable angina, recent surgery or myocardial infarction, cardiac disease with ventricular ejection fraction <30% according to the reports of echocardiography examinations and cancer), limitations of oxygen transport and extraction (severe anaemia and myopathy) or severe walking disability (reported claudication distance <50 m) affecting the capacity to reach the predetermined speed during the treadmill test.

All healthy subjects underwent a clinical examination and were excluded based on the presence of any current or chronic pathological condition. They also underwent an ECD examination performed by the same expert vascular operators.

Echo-colour Doppler report evaluation: On the basis of the ECD report for each participant, legs were classified as diseased or non-diseased. Legs were considered diseased when affected by occlusion, stenosis and/or multiple-vessel atherosclerotic irregularities, even those which did not affect the distal flow.

Ankle–Brachial Index (ABI) measurement: All participants underwent ABI measurement according to the standard21 by means of a Doppler ultrasound device (Stereodop 448.5, Ultrasomed) with a 9.3-MHz probe and a standard blood pressure cuff.

Severity of the disease was ranked on the basis of the following ABI values: normal: 1–1.3; borderline 0.80—0.99; moderate 0.50—0.79; severe: <0.50. Vessels were considered ‘not compressible’ when ABI > 1.40 or when the procedure was interrupted at cuff pressures of 300 mmHg in presence of painful symptoms, even if the Doppler signal at the ankle could not be suppressed.

NIRS measurement and testing procedure: NIRS measurements were obtained with a continuous wave system (Oxymon MK III Artinis Medical System, the Netherlands). The system, consisting of two channels (two equal pulsed light sources, two detectors avalanche photo diode and ambient light protection), uses intensity-modulated light at a frequency of 1 MHz and laser diodes at three wavelengths (905, 850 and 770 nm) corresponding to high absorption of oxyhaemoglobin (O₂Hb) and deoxyhaemoglobin (HHb), with a power auto sensing 110–240 V (approximately 40 Watt). The light of laser diodes is conducted from the instrument to the tissue and back with optical glass fibres 3 m long. When near-infrared light propagates through biological tissue, it is partly absorbed or scattered by the tissues and partly re-collected by the detector. The intensity of the re-collected light provides information about O₂Hb and HHb concentrations as determined by spectral analysis. Additional parameters calculated are the total haemoglobin (THb) (the sum of O₂Hb and HHb) and the difference between O₂Hb and HHb (dHHb).

Skinfolds were measured at the calf by plicometry, and NIRS sensors were then placed and secured with tape along the medial side of the calf (medial gastrocnemius muscle). The interoptode distance was maintained at 4 cm. Each patient was fitted with a heart rate monitor (Sport Tester, Polar Electro, Finland).

Testing was done in the morning in a temperature-controlled environment. The treadmill test used to measure patients’ performance was validated for patients with PAD and based on level walking.22 The test was preceded by
a 1-min warm-up at a speed of 1.5 km h\(^{-1}\), began at 1.5 km h\(^{-1}\); treadmill speed was increased progressively by 0.1 km h\(^{-1}\) every 10 m. The test ended when the patient was unable to maintain the walking speed imposed for any reason (fatigue, dyspnoea and claudication); this was noted as maximal walking treadmill speed. Pain threshold speed was also recorded when a claudication symptom was reported.

The patient’s heart rate (Hr) was recorded at rest in a standing position and at the end of each test fraction. The difference (dHr) between Hr recorded at 1.7 and at 3.0 km h\(^{-1}\) was calculated.

Operators that performed NIRS measurements were blinded to the results of all other measurements. Investigators performing ABI and assisting the patients during the treadmill test execution were not blinded to ECD reports.

Semiquantitative data obtained by the NIRS instrument during the incremental test were analysed using the software Oxyssoft 47. Data within the range 1.7–3.0 km h\(^{-1}\) were transferred into an electronic spreadsheet, (Microsoft Excel 7.0) and, after normalisation to zero, analysed by statistical software (MedCalc 8.0, Medcalc Software, Mariakerke, Belgium) in order to calculate the area-under-curve (AUC) and quantify the individual degree of variation for \(\text{HbO}_2\), \(\text{Hb}\), \(\text{tHb}\) and \(\text{dHb}\) (\(\text{O}_2\text{HbAUC}\), \(\text{HHbAUC}\), \(\text{tHbAUC}\) and \(\text{dHbAUC}\)).

### Statistical analysis

Continuous variables are expressed as mean ± SD or median (range) according to normal or non-normal distribution, and categorial variables as a percentage. The normal distribution of data was verified by the Kolmogorov–Smirnov test.

The characteristics of the two groups of participants were compared using unpaired Student’s t-tests. Differences between groups considered in the various phases of the study were assessed by Mann–Whitney U tests, Kruskal–Wallis tests, or unpaired Student’s t-tests, as appropriate. A Spearman rank correlation was conducted to evaluate the relationship between metabolic parameters and ABI. A stepwise multivariate regression analysis was also assessed to determine whether factors potentially affecting the treadmill performance and/or the energetic cost of walking (age, BMI and ABI) were independently associated with metabolic measurements.

Receiver-operating characteristic (ROC) curves were calculated to identify potential AUC cut-off values for NIRS-based parameters consistent with the presence of multiple vascular atherosclerotic lesions but which were not haemodynamically significant. ECD was considered as a gold standard and the written reports of the vascular operators were analysed. We also considered those legs affected by multiple-vessel atherosclerotic irregularities as diseased, even if the atherosclerotic irregularities did not affect the distal flow.

A p-value of 0.05 or less was considered statistically significant. Data were analysed using the software program Medcalc 8.0 (Medcalc Software, Mariakerke, Belgium).

### Results

Nine out of 76 PAD patients were excluded for the following reasons: unstable angina and low ventricular ejection fraction (\(n = 3\)); myasthenia gravis (\(n = 1\)); cancer under chemotherapy (\(n = 1\)); severe anaemia (\(n = 1\)) and severe walking impairment in chronic stroke (\(n = 2\)).

Sixty-seven PAD patients (56 males, 11 females, age 71.6 ± 8.7 years) were finally enrolled into the study. All healthy subjects recruited (\(n = 28\); 12 male, 16 female; age 30.4 ± 11.9) were also enrolled into the study. The characteristics of the participants are reported in Table 1.

The skin folds at the calf were less than 6 mm for all patients. Healthy subjects were significantly younger than PAD subjects.

The total final sample consisted of 129 diseased and 61 non-diseased legs, five of them belonging to PAD patients but free from any vessel atherosclerotic lesion. Analyses of the two groups were performed according to the phases of the study.

All patients completed the test (maximal walking treadmill speed = 4.1 ± 0.8 km h\(^{-1}\)); 60 PAD patients interrupted the test for claudication symptoms (pain threshold speed = 3.0 ± 0.8 km h\(^{-1}\)) and seven patients for fatigue and dyspnoea in absence of peripheral symptoms. Healthy subjects reached the maximal speed attainable on the treadmill in absence of symptoms or signs of discomfort (maximal walking treadmill speed = 6.8 ± 0.8 km h\(^{-1}\)).

No local or general adverse effects were reported during NIRS measurement.

### Table 1: Characteristics of study participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PAD patients ((n = 67))</th>
<th>Healthy subjects ((n = 28))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>71.6 ± 8.7</td>
<td>30.4 ± 11.9</td>
</tr>
<tr>
<td>Male sex, No. (%)</td>
<td>56 (87)</td>
<td>12 (43)</td>
</tr>
<tr>
<td>Lesion location No (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorto-iliac</td>
<td>12 (18)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fem-pop</td>
<td>52 (78)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Infra-pop</td>
<td>14 (21)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Multiple</td>
<td>8 (12)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Risk Factors, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>23 (34)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>44 (66)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>43 (64)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Smoking</td>
<td>55 (82)</td>
<td>5 (18)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>7 (10)</td>
<td>5 (18)</td>
</tr>
<tr>
<td>Familiarity</td>
<td>20 (30)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Co-morbidities, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>12 (18)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>17 (25)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>4 (6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lung disease</td>
<td>2 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lower limb revascularisation</td>
<td>17 (25)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Therapy, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>5 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Antiaggregants</td>
<td>62 (93)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Statins</td>
<td>43 (64)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>44 (66)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>14 (21)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Continuous variables: mean ± SD. Categorical variables: number (percentage). BMI = Body Mass Index.
Figure 1  The procedures to collect and analyse data by incremental test with NIRS probes within the range 1.7–3.0 Km/h (a) in a healthy subject (H) and in a PAD patient (P) are shown. Semiquantitative NIRS values after normalisation to zero (b) are analysed by statistical software. The area-under the curve (AUC) (c) quantifies the individual degree of variation for oxyhaemoglobin (HbO₂).

Table 2  Values of ABI (Ankle–Brachial Index) and metabolic parameters expressed as area-under-curve (AUC) for diseased vs. non-diseased legs.

<table>
<thead>
<tr>
<th></th>
<th>Diseased legs (n = 129)</th>
<th>Non-diseased legs (n = 61)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI</td>
<td>0.68 (0.30–0.90)</td>
<td>1.11 (1.0–1.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>O₂Hb_AUC</td>
<td>−229 (−1733 to 590)</td>
<td>170 (−272 to 1466)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HHb_AUC</td>
<td>435 (−381 to 2117)</td>
<td>40 (−804 to 404)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dHb_AUC</td>
<td>−638 (−3290 to 826)</td>
<td>159 (−435 to 1704)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>tHb_AUC</td>
<td>217 (−452 to 1730)</td>
<td>270 (−485 to 1373)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

O₂Hb, oxyhaemoglobin; HHb, deoxyhaemoglobin; DHb, difference (HbO₂ − HHbO₂); tHb, total (HbO₂ + HHbO₂). Values are expressed as median (range). P-value, Mann–Whitney U test.
Dynamic NIRS-based measurements in diseased vs. non-diseased legs

An example of the procedure to collect and analyse data is shown in Fig. 1. O$_2$Hb$_{AUC}$, HHb$_{AUC}$ and dHb$_{AUC}$ differed significantly for diseased and non-diseased legs, but tHb$_{AUC}$ did not (Table 2).

Dynamic NIRS-based measurements and severity of disease

Considering diseased and non-diseased legs as a whole and excluding legs with uncompressible vessels ($n = 5$), a significant relationship was observed between O$_2$Hb$_{AUC}$, HHb$_{AUC}$, dHb$_{AUC}$ and the corresponding ABI (respectively: rho = 0.566, rho = 0.521 and rho = 0.599, $P < 0.0001$).

In a subsequent stepwise multiple regression that took into account as dependent parameters O$_2$Hb$_{AUC}$, HHb$_{AUC}$ and dHb$_{AUC}$, among different parameters considered (age, BMI and ABI), only ABI was included as an independent parameter (respectively: $R^2$ = 0.442, $R^2$ = 0.320, $R^2$ = 0.430, $P < 0.0001$). Considering legs of PAD patients and healthy subjects for all parameters but tHb, a different pattern ($P < 0.0001$) was observed in relation to severity of the disease (Fig. 2).

Dynamic NIRS-based measurements and presence of disease

Compared to the ECD positivity, even in terms of multiple-vessel irregularities, the ROC analysis of dHb$_{AUC}$ showed an area of 0.932 (95% CI: 0.886–0.963, $P = 0.0001$) with sensitivity/specificity of 87.6/93.4 for values $\leq$197 (LR + LR – of 13.36 and 0.13, respectively). Significant, but lower values were also observed for O$_2$Hb$_{AUC}$, with an ROC area of 0.910 (95% CI: 0.860–0.947, $P = 0.0001$) and sensitivity/specificity of 78.3/95 for values $>76$ (LR + LR – of 15.92 and 0.23, respectively). The HHb$_{AUC}$ values had an ROC area of 0.861 (95% CI: 0.803–0.906, $P = 0.0001$) with sensitivity/specificity of 71.3/91.8 for values $>280$ (LR + LR – of 8.70 and 0.31, respectively) (Fig. 3).

Dynamic NIRS-based measurements and cardiovascular response in PAD patients vs. healthy subjects

Hr at rest in standing position and at the end of the first fraction (1.5 km h$^{-1}$) was not different between PAD patients and healthy subjects (74 ± 14 vs. 76 ± 11 b min$^{-1}$, 81 ± 12 vs. 82 ± 10 b min$^{-1}$, $P$ = ns) while dHr was

(Kruskal–Wallis test $P < 0.0001$) for all parameters except for tHb$_{AUC}$. Post-hoc analysis, different ($P < 0.05$) from factor n, as follows: (1) Healthy:(3)(4)(5); (2) Normal$_{PAD}$:(3)(4)(5); (3) Borderline$_{PAD}$:(1)(2)(4)(5); (4) Moderate$_{PAD}$:(1)(2)(3); (5) Severe$_{PAD}$:(1)(2)(3). Healthy: healthy legs of healthy subjects; Normal$_{PAD}$: healthy legs of PAD patients (ECD negative, Ankle–Brachial Index: 1–1.3); Borderline$_{PAD}$: legs with Ankle–Brachial Index 0.80–0.99; Moderate$_{PAD}$: legs with Ankle–Brachial Index 0.50–0.79; Severe$_{PAD}$: legs with Ankle–Brachial Index <0.50.

![Figure 2](image-url)
significantly higher in PAD patients ($13 \pm 7$ vs. $6 \pm 4$ b min$^{-1}$, $P < 0.0001$). $dHr$ was not significantly different ($P = 0.10$) between PAD patients with ($n = 14$) and without beta-blocker therapy ($n = 53$).

**Discussion**

With the use of a standardised dynamic NIRS-based test, we detected the degree of muscle deoxygenation for the legs of each subject that was found to be related to the severity of the disease. We quantified this parameter to yield an absolute value and established a cut-off for each NIRS-based parameter consistent with the presence of multiple vascular atherosclerotic lesions, despite a lack of haemodynamic significance, according to the ECD evaluation.

Traditional exercise testing based on the subjective patient’s reported onset of symptoms can be affected by neuropathy, cognitive disorders, compliance or other factors.

Since the 1990s, an abnormal decrease in muscle oxygenation in patients with PAD has been observed when the calf oxygenation of patients with claudication was evaluated dynamically during exercise.$^{13-18}$ However, static or post-exercise measurements (e.g., muscle oxygen consumption or tissue post-hypoxia re-saturation rates) were more often assessed by NIRS.$^{19}$ NIRS is not widely used for PAD patient dynamic evaluation,$^{19}$ despite its objective assessment of muscle metabolism and its possible usefulness for dynamic tests in which walking performance is the result of different factors. Such factors may include cardiovascular system activity, collateral circulation and peripheral extraction, all of which are able to compensate for reduced oxygen delivery.

In order to propose a dynamic objective NIRS-based assessment in a clinical setting, we tested a number of subjects by means of a validated level-walking treadmill test,$^{22}$ after attaching the NIRS probes at the calf muscle. We transformed the individual degree of variation for each NIRS-based parameter into a score by calculating the area-under-curve of the NIRS trace. To obtain values comparable among subjects, we considered data within a predetermined range of exercise intensity. The lower limit was set at $1.7 \text{ km h}^{-1}$, 40 s after the start of the test to eliminate the typical and variable early peak of $O_2$ saturation$^{10}$ (Fig. 2a). The upper limit was fixed at $3.0 \text{ km h}^{-1}$, a speed that most PAD patients can attain. It also represents a sub-maximal walking speed per age, as well a starting level for validated tests in claudication. By means of this method, at a speed at which most patients were still asymptomatic, a different AUC for all parameters but $tHb$ was found between diseased and non-diseased legs. Such different metabolic patterns were related only to the parameter reflecting the $O_2$ delivery (ABI value). However, within the same level of disease, a wide range of metabolic patterns is observable (Fig. 2). We can hypothesise that under dynamic

**Figure 3** Receiver-operating characteristic (ROC) curve of (a) oxyhaemoglobin ($HbO_2$), (b) deoxyhaemoglobin ($HHbO_2$), and (c) of their differential values ($dHb = HbO_2 - HHbO_2$) compared to the ECD positivity for the presence of vessel irregularities, even those not haemodynamically significant.
conditions the muscle oxygenation might be influenced by different factors such as collaterals, peripheral 
O₂ extraction, cardiac function, inotropic and chronotropic response to therapeutic agents, vessel wall stiffness and presence of 
co-morbidities. These factors might be relevant even at 
borderline levels of disease, where the metabolic pattern is 
significantly altered in comparison to healthy legs, within 
a low range of speeds (Fig. 2). On the other hand, a parameter that showed similar levels in both PAD and in 
healthy subjects was tHbAUC. In this test, which takes 
a dynamic picture in an open system where peripheral 
metabolism and cardiovascular function contribute to 
walking performance, we also studied the heart rate 
response. The rate of muscle oxygen use and the cardiac 
output are determinant factors for oxygen delivery.23 
Previous studies reported a reduced heart rate at peak 
exercise in heart failure patients compared with normal 
subjects8 and an enhanced cardiovascular response to 
exercise in patients with metabolic myopathies.12 In our 
study, the heart rate response was crucial for PAD patients 
at an early stage of exercise, with a twofold higher heart 
rate increase at low walking speed compared to healthy 
subjects, an effect that was independent of beta-blocker 
therapy. This compensatory response might explain the 
similar level of local blood volume in the calf, described by 
the parameter tHbAUC, which was observed in legs with and 
without disease.

We also observed that the NIRS-based assessment 
proposed for patients with PAD allowed for good discrimi-
nation of the legs with atherosclerotic lesions that were not 
haemodynamically significant according to the ECD exami-
nation, revealing an abnormal dynamic response. The 
parameter with the highest diagnostic capacity was dHbAUC, 
which reflects both local perfusion and deoxygenation.

In a previous study, despite reproducible measurements, 
NIRS was not considered useful as a diagnostic tool in 
routine clinical practice, when compared to easy, inex-
\[\text{pensive and accurate available methods, such as ABI or toe systolic blood pressure.}^{24}\] However, the proposed NIRS-
\[\text{based method does not aim to substitute, but rather to complete the diagnostic offering in PAD, with the potential to simultaneously evaluate the effects of limiting or compensatory factors on muscle perfusion.}^{25}\]

We are aware that this preliminary study has several 
limitations. The gold standard for PAD diagnosis was ECD, 
which is known to have lower sensitivity—specificity than 
other observed diagnostic techniques.25 Four different 
diagnostic tests were included in the study, each at a stage of exploration, with two parameters that differed according to the 
presence and degree of PAD. The test might be particularly 
useful in a clinical setting to exclude vascular diseases in 
patients with exertional leg pain without the classic clau-
dication symptoms in presence of high and unreliable ABI 
for vessel calcification or borderline ABI values. In these 
patients, the vascular disease and the metabolic deficit 
might be unacknowledged or underestimated.

Further studies will enable us to evaluate the repro-
ducibility of the dynamic NIRS-based test, to evaluate its 
diagnostic capacity on a large sample of asymptomatic and 
symptomatic people and to observe the effects of training on 
these proposed metabolic parameters.

Conflict of Interest

The authors have no conflict of interest.

References

2. Belardinelli R, Barstow T, Porszasz J, Wasserman K. Changes in skeletal muscle oxygenation during incremental exercise 
6. Colier WNJ, Meeuwsen IB, Degens H, Oeseburg B. Determination of oxygen consumption in muscle during exercise using 
11. Hamaoa T, McCully KK, Quaresima V, Yamamoto K, Chance B. Near-infrared spectroscopy/imaging for monitoring muscle 


