A Near Infrared Spectroscopy-Based Test of Calf Muscle Function in Patients with Peripheral Arterial Disease

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Abstract

Background The study aims to test a new, simple, and reliable apparatus and procedure for the diagnostics and treatment evaluation of peripheral arterial disease (PAD). The test apparatus involves near infrared spectroscopy (NIRS) of a main part of the lower leg muscles during isometric flexion and extension of the ankle joint performed with the foot strapped in a specially designed pedal ergometer.

Design To evaluate the reproducibility of the new test compared with an existing testing method of treadmill walking.

Methods Eleven patients participated in the study: nine patients with claudication and two age-matched patients without claudication. Each patient was tested with an isometric ergometer pedal test and a treadmill test applying NIRS measurements of the anterior tibial and the gastrocnemius muscles (GAS). Tests were repeated three times with randomly selected intervals between individual test runs. Intraclass correlation constant (ICC) was used to describe reproducibility. The ICC was calculated using the area under the NIRS oxygenated hemoglobin (Hb\textsubscript{oxy}) curve, the initial velocity of the Hb\textsubscript{oxy} recovery curve, force measurements, and walking time.

Results The ICC of the GAS was between 0.92–0.95 (foot-pedal) and 0.70–0.98 (treadmill) and of the anterior tibial muscle was between 0.87–0.96 (foot-pedal) and 0.67–0.79 (treadmill).

Conclusion In this study, we contribute a new apparatus and test protocol for peripheral arterial disease (PAD) applying NIRS technique and controlled physical activity to evaluate the degree of muscle oxygenation under specific functionally relevant conditions. Thus, we have developed a clinically applicable “easy-to-do” exercise test of patients with chronic PAD which show high reproducibility.

The objective of this study was to evaluate whether a new simple isometric near infrared spectroscopy (NIRS) foot pedal test of lower leg muscles could be used for functional testing of patients suffering from peripheral arterial disease (PAD) in a daily clinical setting as an alternative to the treadmill test now being used. In a comparison of the new test and the existing treadmill test, we applied NIRS recording in both tests. We hypothesized that a pedal test would have an equal or better reproducibility than a standard treadmill test because the pedal test was designed to reduce factors that influence the reproducibility.

The diagnosis and clinical staging of PAD is currently based on patient symptoms of muscle ischemia combined with ankle blood pressure (ankle brachial index [ABI]),
angiography, magnetic resonance angiography, computer tomography angiography, ultrasound duplex scanning, and other clinical findings. Unfortunately, the test results with these procedures are not always specific with respect to patient symptoms and clinical findings. This is most likely the result of different pain thresholds and perhaps also different daily-life activity levels among patients. The reported symptoms, such as reduced walking distance in claudication, are known to involve major intraindividual as well as interindvidual variability and are not always a true reflection of the actual walking distance. In particular, in patient cases with claudication symptoms and a normal ABI, the need of a more appropriate functional exercise test has become apparent.

Existing Functional Test of Peripheral Arterial Disease

The existing functional tests of PAD involve either whole body level, such as a treadmill walking test, or isolated muscle group exercise of the lower extremities, such as the new pedal test described in this article. Both tests are conducted with measurements of pre- and postexercise ABI.

The Treadmill Test

Using whole body exercise-based tests introduces several problems. One problem is reduced walking distance/exercise time by dysfunctions other than leg claudication, for example, cardiac angina, chronic obstructive lung disease, and mechanical disabilities of the legs, which was pointed out by Yamamoto et al. In situations where other morbidities coexist with PAD, walking time will not be diagnostic specific of PAD because walking time/distance is also reduced by other symptoms than claudication. Nevertheless the treadmill could provide valuable information on what actually stops the patient from walking, the comorbidity or PAD. But this is difficult in situations where both diseases contribute equally to the walking disability. Treadmill testing is often impossible to perform in the practical clinical setting in patients with critical ischemia since most of them are typically not able to walk. Nevertheless, the treadmill test is probably the test procedure which most closely mimics the situation of real walking.

The Pedal Tests

With single muscle group exercise, such as a pedal test, some of the problems with the interference by other diseases could be avoided and the test, therefore, most directly evaluates the relation between muscle activity and blood supply. In the pedal test, this is achieved with the patient in a seated position and by reducing the strength of the muscle work which will reduce the influence of common comorbidities, such as, chronic obstructive pulmonary disease, cardiac angina, and mechanical disabilities.

This study presents a new apparatus and test. Various dynamic pedal protocols have been presented before, that is, Yamamoto et al and Cameron et al, both of whom used a test based upon pain evaluation during exercise. Yamamoto found reliability of 0.92 (Cronbach α value) for the same treadmill protocol as used in this study and 0.90 for a foot pedal test. Both values were obtained for maximal walking distance/exercise time. Lower reproducibility was found using pain-free walking and pain-free exercise time (0.88 and 0.66). NIRS was used in both tests although not reported in detail. Manning et al did not report reliability values but concluded that a dynamic pedal test with fixed duration (2 minutes/exercise frequency 60 per minute) had a higher sensitivity for PAD compared with a treadmill test. NIRS was not used in these studies and actually 33% of the patients did not produce a decrease in the post ABI measurements with the pedal test.

The above-mentioned studies reported less reproducibility in the situation with pain-free exercise and, therefore, we chose an isometric exercise protocol with fixed exercise duration of only 1 minute with maximal voluntary contraction (MVC) to insure a significant decrease in the NIRS parameters. This way we attempted to make the new test more clinically useful and less time consuming.

Near Infrared Spectroscopy Measurements

Measuring the ABI pre- and postexercise is time consuming and often there is a time delay between the end of the exercise and the postexercise ABI values, depending on the experience of the investigator, which certainly contributes to the reliability of the test.

NIRS measures oxygenation continuously in the relevant muscle noninvasively, which is a much simpler technology to implement compared with the ABI-based protocols. The actual technique works by emitting light by a laser optode in the near infrared spectrum (800–900 nm) through the skin and into the underlying muscle. The light is penetrating the tissue in all directions and is absorbed specifically by hemo-globin in the blood and myoglobin in the muscle. The receiving optode collects a minor fraction of the emitted light and the amount of light received depends primarily on the distance between the two optodes and the concentration of the specifically absorbing molecules in the blood and tissue. The difference in the absorbance of light of different wavelength is exploited to determine the relative oxygenation level of the measured tissue, since oxygenized and deoxygenized hemoglobin have different absorption spectra. The major part of the light received by the receiving optode will have traveled the shortest distance from the emitter to the receiver optode and in practical terms the penetration depth from which the signal is received is, roughly speaking, half the length of the distance between the light emitter and sensor (approximately 2 cm in this study). Measuring muscle oxygenation provides the balance between actual perfusion and muscle oxygen consumption. It is commonly known that muscle oxygen consumption depends on the muscle fitness of the patient. Therefore, NIRS measurement, in contradiction to ABI measurement, takes muscle fitness into account. Muscle fitness is one of the major factors contributing to the wide range of symptoms seen at any given resting ABI. Muscle mitochondrial function is also an important factor, especially in PAD where a reduction in mitochondrial function is seen.

Treadmill NIRS measurements obtained in the gastrocne-mius muscle (GAS) have previously been used on patients
with PAD. These studies have shown high reproducibility and a high correlation with the degree of PAD.\textsuperscript{8,9} NIRS during treadmill testing has been shown to correlate with measurement of ABI.\textsuperscript{9}

Testing of muscle force and oxygenation with a pedal and NIRS has been done earlier in several studies\textsuperscript{3,10,11} and a reduced cardiac stress compared with the treadmill test in patients with PAD has been shown.\textsuperscript{3,12}

With this new test, we hope to be able to include muscular fitness into the diagnostics of PAD in a simple clinical applicable way instead of just looking at the blood pressures. It was our aim to develop a test that can give a more precise diagnosis and the degree of disease.

**Methods**

Patients: Eleven patients (seven men and four women) were recruited from the vascular outpatient clinic at Gentofte Hospital, Denmark, nine with claudication and two age-matched patients without claudication, for details see Table 1. The distribution of the different patient types was chosen to mimic the normal distribution of referred patients in our outpatient clinic. PAD was defined according to the TASC II consensus report.\textsuperscript{1} All patients were also enrolled in a larger patient series with the purpose of investigating muscle energy metabolism in patients with combined PAD and diabetes type 2. Only reproducibility of measurements and feasibility of the pedal test are considered in this article.

In addition, a series of tests was done using one of the authors (BLP) as a subject to get a picture of reproducibility under the most optimal conditions.

Oral and written informed consent was obtained from all patients and the project was approved by the local ethical committee (approval nr: H-D-2007–0074).

All patients with claudication were nonsurgically treated at the time of investigation, following a program with exercise counseling and medical treatment with antiplatelet and cholesterol-lowering drugs (Table 1). The pedal-based exercise test was developed and compared with an existing test based upon the recording of walking distance on a treadmill. The pedal test and the treadmill test were performed on the same day with appropriate recovery time in between. Both tests were repeated three times on three different randomly selected days with a mean time of 22.6 days (mean, range 2–130) in between. No changes in exercise pattern of the patients were detected in the investigation period. All tests were done by the primary investigator.

The ergometer pedal instrument: The developed pedal instrument is based upon a pedal ergometer designed for a whole-body magnetic resonance scanner.\textsuperscript{13}

The pedal is made of black polyoxymethylene (POM) plastic with a sole of soft material which follows the shape of the foot and is adjustable to both the feet and leg length. The pedal is attached to a base of hard plastic which again is fixed to a hospital examination bed with wheels and stabilizing legs to avoid unnecessary movement. The foot is attached with the Velcro straps. Between the rear side of the pedal and the pedal base, a brass stick with an incorporated strain gauge

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics and medication</th>
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<tbody>
<tr>
<td><strong>Diabetes type 2</strong></td>
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<tr>
<td>---------------------</td>
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<tr>
<td>PAD</td>
</tr>
<tr>
<td>No PAD</td>
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<tr>
<td><strong>Medication</strong></td>
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<tr>
<td>Cholesterol-lowering drugs</td>
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<tr>
<td>β-Blocking drugs</td>
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<tr>
<td>Angiotensin antagonists</td>
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<tr>
<td>Calcium antagonists</td>
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<tr>
<td>β2-Agonists</td>
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<tr>
<td>Oral antidiabetics</td>
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<tr>
<td>Anticoagulants</td>
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<tr>
<td>Other</td>
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</tbody>
</table>

**Abbreviations:** ABI, ankle brachial index; BMI, body mass index; PAD, peripheral arterial disease; SD, standard deviation.
The walking speed was set at 2.4 km/h with an inclination of 12% after an initial resting period of 3 minutes as previously done. The patients continued to walk until the calf pain made them discontinue or the tests were terminated after 5 minutes (distance 200 m). Measurements continued afterwards for a period of 6 to 10 minutes until the NIRS parameters had recovered.

The experimental setup is shown in Fig. 1 and an example of both tests is shown in Figs. 2 and 3.

Calculations and Statistics

All fittings and graphs were made using GraphPad Prism 5 (San Diego, CA).

The area under the Hbox curve was used to compare the oxygen decrease during exercise. The initial slope of the recovery curve was determined using a linear regression model to describe the initial velocity of the recovery of Hbox, like previously done by Kemp et al. The kinetics of the NIRS–Hbox recovery curve has a fairly constant initial velocity until reaching a plateau. In some cases, an exponential fit was more suitable but unfortunately this is not always the case. Some patients have different recovery curve shapes according to the degree of disease, which makes a linear model more suitable. The intercept with the x-axis of the fitted line is the assumed recovery time. Reliability for the recovery periods in both exercise tests was obtained by comparing the slopes of the recovery linear fit.

The force measurements obtained during pedal exercise were expressed as impulse (N × s), which is a surrogate for workload in isometric exercise. The impulse was calculated using the voltage output from the strain gauge calibrated with a 10 kg weight multiplied with the exercise duration in seconds and the acceleration of gravity (mean kg in the exercise period × 60 seconds × 9.807 m/s² = impulse Ns.)
One way, ANOVA and ICC were used to describe reproducibility. The ICC is a way to incorporate both within and between people variability. Statistical significance was defined as a $p$-value $< 0.05$. All statistics were done using SPSS 20.0 (IBM, Chicago, IL).

**Results**

In all patients and controls, a decrease in Hb$_{ox}$ was observed during exercise in both muscles. An increase in Hb$_{deox}$ during exercise was seen for all patients in the pedal test. In the treadmill protocol, the same response was true for patients with PAD, but in the experiments on the controls, including one of the authors (B.L.P.), it either decreased in the GAS muscle or stabilized on a low level in the TA muscle (see Figs. 2, 3, and 4 for experiment examples).

The ICC of the GA muscle was between 0.92–0.95 (foot-pedal) and 0.70–0.98 (treadmill) and the anterior tibial muscle was between 0.87–0.96 (foot-pedal) and 0.67–0.79 (treadmill) (Table 2). Reliability using ICC values and other

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**Fig. 2** Control experiment with the pedal test using one of the authors (B.L.P.) as a subject (each curve consists of six replicates).
reliability values, for example, Cronbach’s α value, is generally considered excellent > 0.80 and fair > 0.60. This places the values obtained in this study in the high end of the scale for both tests.\(^3\,^8\)

Generally, reproducibility was lower in the treadmill test compared with the pedal test in both the exercise and recovery situations, both due to wide 95% confidence intervals of the ICC values in the treadmill test no significant (\(p > 0.05\)) difference could be established. The reliability of the walking time was high but is lower in reality because the treadmill protocol was terminated at 200 m. The reliability of muscle force measured as impulse was high in the pedal test. No difference in the reproducibility was found between the GAS and the TA muscles for the pedal test except for a slightly lower ICC for the TA muscle in the force measurements, although not significant. In the treadmill test, a lower ICC was seen in the exercise period for the TA muscle but was opposite in the recovery period. The ICC reliability results are summarized in Table 2 and the Hbox data and calculated kinetics are shown in Table 3.

**Discussion**

The findings of this study show superior reproducibility of the isometric NIRS pedal test compared with the NIRS treadmill tests (ICC GAS muscle 0.92–0.95 [foot-pedal] and 0.70–0.98 [treadmill], TA muscle 0.87–0.96 [foot-pedal] and 0.67–0.79 [treadmill]), even though no significant difference is detected.

![Fig. 3](image)
because of the large confidence intervals of the treadmill test. But this fact in itself indicates a wider range of measures in the treadmill test. This is likely to be a reflection of factors described in the next section.

**Area under the Curve**

The difference in ICC values between the pedal test and the treadmill test in this parameter is seen in the TA muscle where the difference is approximately 15% opposed to the GAS muscle where it was only 8% (Table 2). This difference could be explained by a smaller decrease in Hb<sub>ox</sub> in the TA muscle during walking compared with the isometric exercise which could compromise reproducibility.

The use of area under the decreasing Hb<sub>ox</sub> NIRS curve (AUC) in the exercise period was considered as a fair method to describe reproducibility. Another method using the initial velocity of the oxygen decrease during exercise could have been used. But AUC was chosen because it omits the exercise duration where the initial velocity does not.

**The Near Infrared Spectroscopy Recovery Curve**

Generally, the NIRS recovery kinetics reflects the quality of the revascularization after exercise. The difference in ICC values between the two tests was still highest in the pedal test for both muscles, but during recovery the highest difference was seen in the GAS muscles (15%) and the lowest in the TA muscles (6%), which is the opposite response compared with the AUC during exercise. The explanation for this might be the same as for the AUC curve, but it is not clear.

The interpretation of recovery curve in the treadmill test in patients without PAD is somewhat different compared to...
patients with PAD, because some of these patients have already recovered their HbO₂ within the exercise period. This is explained by the compensatory blood flow response to exercise.\textsuperscript{15} In patients with PAD, the blood flow response is impaired due to the stiffness of the vascular bed which makes the vessels less compliant and, consequently, the normal blood flow response to exercise is insufficient.\textsuperscript{16} In patients with PAD, the recovery curve never recovers within the exercise period on the treadmill. If a patient with PAD has a more horizontal slope of the recovery curve, it means that they will have a longer recovery time and hereby be more severely affected by their disease.\textsuperscript{8} If a person without PAD has a horizontal slope of their recovery curve it will have the opposite meaning. However, this phenomenon is not seen when using the pedal test. The pedal test is, therefore, properly more feasible to differentiate between mild and no claudication. The reproducibility of the NIRS treadmill test was lower in this study compared with results achieved by, for example, Komiyama who reported ICC values of 0.92 in 21 legs in 11 patients. Komiyama et al had a narrower range in intervals between measurements which was 6 days (mean, range 3–9 days). Interval between measurements was 22.6 days (mean, range 2–130 days) in this study and could explain the lower reproducibility; however, if this was the case one would have expected the same low reproducibility for the pedal test, which clearly was not the case. Komiyama\textsuperscript{8} used recovery time and not initial recovery velocity in this study which could again explain the difference.

### Muscle Force and Walking Time

These two parameters are an expression of the amount of muscle work done in the two tests. Both show high reproducibility values. The highest is seen in the walking time but these values may be falsely elevated because of the termination of the treadmill test, which is set at 200 m.

Equal ICC values are seen between the two muscle types in both tests.

### Body Mass Index and Physical Fitness

Body mass index (BMI) and physical fitness contribute to the intersubject variability of whole body exercise more than a single-joint exercise. BMI varies among patients involved in this study with a SD of $±18\% (25 ± 4.6)$. Variability of the BMI is not reported in the cited articles involving treadmill exercise. Therefore, the impact of BMI on their results cannot be studied. Whole body physical fitness in this study was not measured and walking time/distance cannot be used as a true reflection of variability because the treadmill test was terminated at 200 m. In general among PAD patients, a coefficient of variability (CV) of 43% was seen in walking distance in a recent study with GPS-monitored walking distance.\textsuperscript{12}

The data obtained with one of the authors (B.L.P.) as a subject were included to illustrate the data with a younger, fit person well acquainted with the instrument and the procedure. These control experiments in fact scored the highest reproducibility (see – Figs. 2 and 4).

### Table 2

<table>
<thead>
<tr>
<th>Pedal instrument</th>
<th>Treadmill</th>
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<tbody>
<tr>
<td>Gastrocnemius</td>
<td>Anterior tibial</td>
</tr>
<tr>
<td>Exercise (AUC)</td>
<td>0.936 0.828–0.981</td>
</tr>
<tr>
<td>Initial recovery velocity</td>
<td>0.953 0.873–0.986</td>
</tr>
<tr>
<td>Walking time</td>
<td></td>
</tr>
<tr>
<td>Muscle force (impulse)</td>
<td>0.920 0.785–0.976</td>
</tr>
</tbody>
</table>

Abbreviation: AUC, area under curve.

### Table 3

Calculated NIRS-kinetics from HbO₂ of all patients ($N = 11$; ± SD)

<table>
<thead>
<tr>
<th>Pedal instrument</th>
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</tr>
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<tbody>
<tr>
<td>Gastrocnemius</td>
<td>Anterior tibial</td>
</tr>
<tr>
<td>Oxy hemoglobin data for all subjects ($n = 11$)</td>
<td></td>
</tr>
<tr>
<td>AUC exercise</td>
<td>302.9 ± 261.8</td>
</tr>
<tr>
<td>Initial recovery velocity</td>
<td>0.25 ± 0.21</td>
</tr>
<tr>
<td>Force (impulse)</td>
<td>13,222.8 ± 6,171.0</td>
</tr>
<tr>
<td>(N x s)</td>
<td></td>
</tr>
<tr>
<td>Walking time</td>
<td>233.3 ± 80.7</td>
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The observed curve patterns between the different parameters of hemoglobin are in concordance with the earlier findings of NIRS experiments of patients with PAD and nondiseased.3,8–11,18 The \( H_{b\text{ox}} \) and \( H_{b\text{deox}} \) curves in the pedal test were a mirror reflection of each other in both the patients with and without PAD, but the span of the patients with PAD was 10 to 15 times higher (Figs. 2 and 3). The \( H_{b\text{total}} \) increased in the controls during exercise but kept constant in the patients with PAD. This could be explained by the stiffness of the large arteries in the leg in the patients with PAD impairing the normal increase in perfusion by arterial dilatation during exercise.13 During recovery, an elevated \( H_{b\text{total}} \) is seen in all patients and is the result of the postischemic hyperaemia,15 which also shifts hemoglobin balance towards a greater amount of \( H_{b\text{ox}} \) compared with \( H_{b\text{deox}} \).

During the treadmill exercise, the response was different. In the experiments using one of the authors, an initial drop in both \( H_{b\text{ox}} \) and \( H_{b\text{deox}} \) was seen in both muscles. The starting level of \( H_{b\text{deox}} \) and \( H_{b\text{total}} \) was high, properly reflecting the pooling of blood in the legs in the standing position.10 In the GAS muscle, a slight increase of \( H_{b\text{ox}} \) and a slight decrease of \( H_{b\text{deox}} \) and \( H_{b\text{total}} \) were seen during exercise. In the TA muscle, all values stabilized on a constant level. During recovery, the same response as for the pedal protocol was seen.

The explanation for the observed difference in \( H_{b\text{ox}} \), \( H_{b\text{deox}} \), and \( H_{b\text{total}} \) between the pedal and treadmill test during exercise should be found in the difference of isometric and dynamic exercise types. In the isometric exercise, the perfusion is blocked during exercise because of the constant contraction in the whole exercise period. In the treadmill test, perfusion is not blocked in the same way because of the intermittent contraction during walking, allowing perfusion during muscle relaxation.

Statistical Evaluation of Reproducibility of Measurements

The use of ICC in this study was chosen as a key parameter because of its common use in previous studies that make the ICC more comparable even though ICC values from different studies are not really comparable unless a similar study design is applied.20,21

The excellent ICC of the pedal test is high compared with the dynamic foot pedal test presented by Yamamoto et al and Manning et al.3,12 No real difference in reproducibility was found between the GAS and TA muscles except for a lower reproducibility for the TA muscle during exercise in the treadmill protocol (Table 2).

The simplicity and usefulness of the pedal test renders it as a candidate of standard test in the PAD clinic as demonstrated in this study demonstrating high reproducibility of PAD evaluation. Thus, it would seem worthwhile investigating the method further on a larger number of PAD patients focusing also on a model for disease sensitivity and specificity. This could be achieved by setting up a randomized study where patients are randomized to a treadmill test or to the isometric pedal test, where the treadmill test is considered the golden standard. Resting ABI could also be chosen as the golden standard. Interobserver agreement should also be considered because all tests were done by the same person in this study. The result of such a study will properly show lower reproducibility like in other clinical tests where several persons perform the same test.22

Calf pain in PAD patients is predominately located in the GAS muscles and almost never in the TA muscles, which also is used during walking and represents the agonistic muscle force to the GAS muscle. Furthermore, the TA muscle as opposed to the GAS muscle is mainly an oxidative muscle in humans containing 69% type I and 27% type IIa fibers.23,24 To elaborate whether the measurement in the TA muscle has equal reproducibility, we tested this muscle.

In summary, the new pedal test shows high reproducibility, are easier to complete for both investigator and patient compared with older ABI-based protocols. The contribution of the BMI on the in between subject variability are reduced. Muscle oxygen consumption is omitted in the determination of the disease stage, which is left out using ankle pressures. Using NIRS, continuous measurements are provided; this is impossible using ABI measurements. Compared with the NIRS treadmill test, the reliability is slightly higher, the pedal test can be used on patients suffering from critical ischemia which the NIRS treadmill test. The pedal test are most likely better at separating muscle ischemia due to PAD from other comorbidities influencing walking distance when these diseases equally effect walking.

Conclusion

This study describes a new, simple pedal apparatus and test designed to evaluate the level of the PAD disease as well as the effects of various treatment procedures applied to these patients. The new pedal test was compared with an existing treadmill test. The pedal test proved easy to carry out and was well tolerated by the patients. Also, the pedal test showed higher reproducibility of test results on the same patient than the treadmill test.

The test procedure developed remains, however, to be applied in a larger group of patients.

Acknowledgments

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