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A low-cost, wireless near-infrared spectroscopy device detects the presence of lower extremity atherosclerosis as measured by computed tomographic angiography and characterizes walking impairment in peripheral artery disease

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ABSTRACT

Background: Patients with peripheral artery disease (PAD) who experience intermittent claudication report a range of symptoms. Patients with symptoms other than classically described intermittent claudication may be at the highest risk for functional decline and mobility loss. Therefore, technologies allowing for characterization of PAD severity are desirable. Near-infrared spectroscopy (NIRS) allows for measurements of muscle heme oxygen saturation (StO₂) during exercise. We hypothesized lower extremities affected by PAD would exhibit distinct NIRS profiles as measured by a low-cost, wireless NIRS device and that NIRS during exercise predicts walking limitation.

Methods: We recruited 40 patients with PAD and 10 control participants. All patients with PAD completed a computed tomographic angiography, 6-minute walk test, and a standardized treadmill test. Controls completed a 540-second treadmill test for comparison. StO₂ measurements were continuously taken from the gastrocnemius during exercise. Variables were analyzed by Fischer's exact, χ^2 , Wilcoxon rank-sum, and Kruskal-Wallis tests as appropriate. Correlations were assessed by partial Spearman correlation coefficients adjusted for occlusive disease pattern. **Results:** Patients with PAD experienced claudication onset at a median of 108 seconds with a median peak walking time of 288 seconds. The baseline StO₂ was similar between PAD and control. The StO₂ of PAD and control participants dropped below baseline at a median of 1 and 104 seconds of exercise, respectively ($P < .0001$). Patients with PAD reached minimum StO₂ earlier than control participants (119 seconds vs 522 seconds, respectively; $P < .001$) and experienced a greater change in StO₂ at 1 minute of exercise (-73.2% vs 8.3%; $P < .0001$) and a greater decrease at minimum exercise StO₂ (-83.4% vs -16.1%; $P < .0001$). For patients with PAD, peak walking time, and 6-minute walking distance correlated with percent change in StO₂ at 1 minute of exercise ($r = -0.76$ and -0.67 , respectively; $P < .001$) and time to minimum StO₂ ($r = 0.79$ and 0.70 , respectively; $P < .0001$). **Conclusions:** In this initial evaluation of a novel, low-cost NIRS device,

lower extremities affected by PAD exhibited characteristic changes in calf muscle StO₂, which differentiated them from healthy controls and were strongly correlated with walking impairment. These findings confirm and expand on previous work demonstrating the potential clinical value of NIRS devices and the need for further research investigating the ability of low-cost NIRS technology to evaluate, diagnose, and monitor treatment response in PAD. (J Vasc Surg 2020;71:946-57.)

Keywords:

Peripheral artery disease; Near-infrared spectroscopy; Six-minute walk; Gardner-skinner protocol

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Peripheral artery disease (PAD) affects 8.5 million individuals over the age of 40 in the United States and more than 200 million worldwide.^{1,2} Patients with PAD experience progressive walking impairment, reduced daily activity, and poor quality of life in conjunction with an increased risk of all-cause mortality.^{1,3-7} A common manifestation of PAD is intermittent claudication (IC). However, patients with IC experience a range of lower extremity symptoms, which can be confused with diseases affecting the back and the musculoskeletal system of the legs.⁸ These patients with atypical presentations may be at the highest risk for functional decline and mobility loss.⁹ Therefore, it is desirable to develop technologies that allow for identification of arterial-related walking impairment, provide insight into the pathophysiology of PAD, and characterize PAD severity.

Near-infrared spectroscopy (NIRS) is a noninvasive technology that allows for dynamic measurements of oxygen-bound hemoglobin and myoglobin in skeletal muscle. By extension, NIRS provides a window into the state of the muscle environment and possible interactions among oxygen delivery, demand, and use. Previous studies using NIRS to evaluate calf muscle heme oxygen saturation (StO₂) have demonstrated that patients with PAD have a greater magnitude of desaturation at peak exercise, and a delay in the recovery to baseline StO₂ after exercise.^{10,11} However, many of these studies used wired NIRS devices that are cost prohibitive outside of the clinical research environment. Currently, there are limited data on the efficacy of wireless, lower cost NIRS devices in the study of PAD.

The specific aims of this present work were twofold. Our first objective was to test the hypothesis that extremities affected by PAD exhibit distinct NIRS profiles that differentiate them from healthy controls as measured by a novel, cost-effective, and wireless NIRS device. Additionally, we sought to evaluate and characterize the relationship between walking limitation and NIRS parameters during exercise testing.

METHODS

Patient recruitment and demographics.

Institutional review board approval was obtained from the Nebraska- Western Iowa Veterans Affairs Medical Center and the University of Nebraska Medical Center. All patients gave informed consent before enrollment. Patients with PAD were recruited from an established vascular surgery clinic. Patients with PAD were eligible for recruitment if they had a history of chronic claudication, exercise-limiting symptoms during a directly observed walking test, a resting ankle-brachial index (ABI) of less than 0.90, and were on a stable blood pressure, lipid, and diabetes regimens for more than 6 weeks. Exclusion criteria included Fontaine stage III or IV disease, acute lower extremity

ischemia from thromboembolic disease; walking capacity limited by a severe comorbid musculoskeletal, neurologic, or cardio-pulmonary disease; and an inability to participate in the described treadmill protocol. Controls were recruited from the community. Control participants were eligible for recruitment if they had no history of ambulatory impairment and were found to have normal pulses, normal resting ABI, and no evidence of claudication in a directly observed walking test. Basic patient demographics including age, sex, race, medical comorbidities, family history of cardiovascular disease, smoking status, and other PAD-related risk factors were documented at the initial visit. To characterize the location and degree of occlusive disease, patients with PAD underwent computed tomographic angiography (CTA), resting ABI, and stress ABI.

Resting and stress ABI measurements.

ABI measurements were performed by a dedicated vascular laboratory technologist. Systolic pressures were taken from the bilateral brachial, dorsalis pedis, and posterior tibial arteries after a period of rest in the supine position. The maximum systolic pressure from the dorsalis pedis or posterior tibial was divided by the highest of the bilateral brachial systolic pressures. After the resting pressure measurements, the blood vessels at the level of the distal thigh were occluded with a pressure cuff for 5 minutes. Occlusion was confirmed by the absence of an ipsilateral Doppler signal in the pedal arteries. After the cuff was released, systolic pressure measurements were repeated at 15-second intervals until the pressure returned to baseline. Minimum stress ABI (at 15 seconds) and percent change from baseline resting ABI were recorded.

CTA.

Patients with PAD underwent CTA with dedicated tibial runoff and delayed imaging. 1.5-mm cross-sectional images were obtained from the abdominal aorta to pedal vessels. Occlusive disease patterns were described as aortoiliac, femoropopliteal, combined aortoiliac þ femoropopliteal, combined femoropopliteal þ tibial disease, and combined aortoiliac þ femoropopliteal þ tibial disease. Tibial disease was further classified by the number and location of involved runoff arteries. The degree of arterial stenosis was considered mild if the arterial diameter was decreased by less than 30%, moderate if the arterial diameter was decreased by 30% to 70%, and severe if the arterial diameter was decreased by more than 70%.

Six-minute walk test.

Patients with PAD performed a 6-minute walking test as previously described.¹² The total 6-minute walking distance (6MWD) was recorded.

Treadmill protocol in PAD and control cohort.

Patients with PAD completed a Gardner treadmill walking test.¹¹ Participants walked on a dynamic gait and pressure analysis treadmill (FDMT SciFit AC5000M, Noraxon USA, Inc., Scottsdale, Ariz), which collects synchronized pressure measurements allowing for gait analysis and the calculation of steps taken during exercise. Participants initiated exercise at 2.0 miles per hour at 0.0% incline. With each subsequent 2-minute stage, the treadmill incline was increased 2.0% to a maximum of 12.0%. Time of initial symptoms (claudication onset time [COT]) and time at which claudication symptoms were prohibitive to further walking (peak walking time [PWT]) were recorded. To allow for comparison across the range of PWTs in the PAD cohort, control participants performed a 540-second Gardner test. This represented the average PWT of a cohort of highly functioning patients with PAD early in the recruitment process.

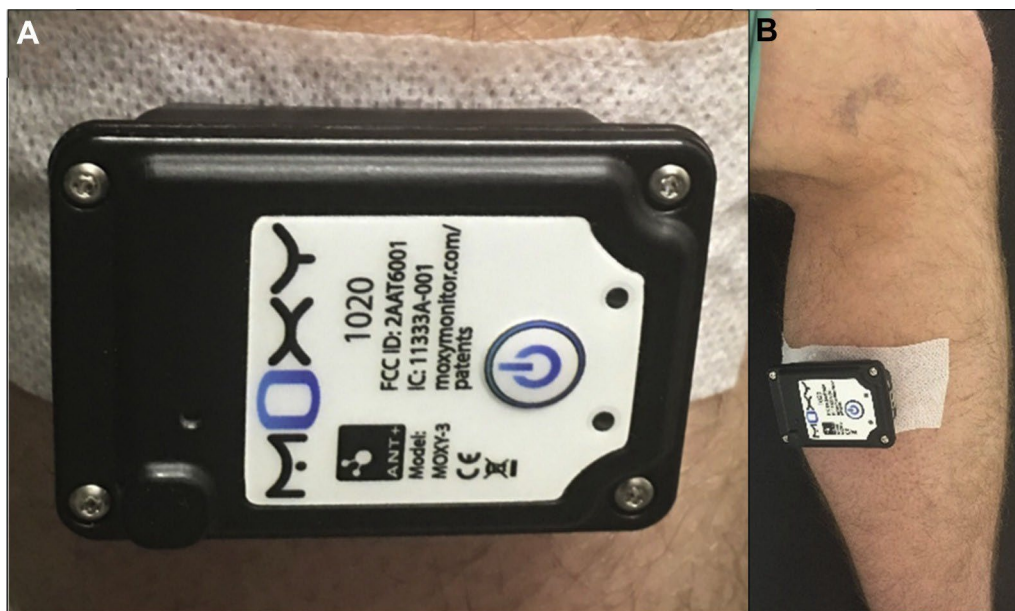


Fig 1. MOXY near-infrared spectrophotometer. The device measures $2.40 \times 1.72 \times 0.82$ inches and houses four laser emitting diodes (wavelengths 680, 720, 760, and 800 nm), two incrementally spaced light detectors, and a lithium polymer battery with a 6-hour battery life. The device is attached to the lower extremity with a dual-sided adhesive tape before securing with Coban. **A**, Close up image of the MOXY NIRS probe. **B**, Application of MOXY to the lateral head of the gastrocnemius.

MOXY near-infrared spectrophotometer. NIRS measurements were taken with a wireless, continuous-wave, near-infrared spectrophotometer (MOXY, Fortiori Design LLC, Hutchinson, Minn). Each device measures $2.40 \times 1.72 \times 0.82$ inches and houses four laser-emitting diodes (wavelengths 680, 720, 760, and 800 nm), two incrementally spaced light detectors, and a lithium polymer battery with a 6-hour battery life.¹³ Measurements can be collected wirelessly in real time or stored on an internal hard drive for future retrieval. The probe noninvasively measures oxygenated and deoxygenated heme-containing molecules (hemoglobin β myoglobin) in the target tissue using a modification of the Beer-Lambert law. The device's StO_2 algorithm controls for multiple confounding factors, including skin/subcutaneous blood flow.¹³ The makers of the device state that they have developed and use these algorithms with the goal of providing a more specific measurement of muscle StO_2 as compared with traditional NIRS devices.¹³

StO_2 represents the percentage of heme-containing molecules that are oxygen bound within the muscle. StO_2 measurements were relayed wirelessly in real time using the software PeriPedal (PeriPedal, Napoleon, Ind). A previous report of the test-retest variability of resting StO_2 measurements using the MOXY device showed a coefficient of variance ranging from 5.7% to 6.2%.¹⁴ This measure is comparable with other commercially available NIRS devices.^{14,15}

Measurement of calf muscle StO_2 during exercise (PAD and control cohort). NIRS monitors were placed over the bilateral gastrocnemius in all PAD and control participants (Fig 1). Seated baseline measurements were taken for a minimum of 3 minutes or until StO_2 signal stabilized, whichever was longer. Measurements were continuously captured during the exercise and recovery interval. The time to drop below baseline resting StO_2 (below baseline time [BBT]) was documented. In addition, specific StO_2 measurements were collected at 1 minute of exercise, namely, the COT and PWT. The minimum achieved StO_2 was also

documented. Thirty minutes of seated recovery data were collected in the PAD cohort. Recovery variables collected included time to 50% recovery of baseline StO₂ (T₅₀), time to 100% recovery of baseline StO₂ (T₁₀₀), average recovery slope, maximum recovery StO₂, and time to maximum StO₂.

Table I. Demographics for PAD and control cohort

	PAD	Control	P value
Total number	40	10	
Male sex	40 (100.0)	10 (100.0)	NS
Race			.013
Caucasian	38 (95.0)	7 (70.0)	
African American	2 (5.0)	0 (0.0)	
Asian	0 (0.0)	2 (20.0)	
Hispanic	0 (0.0)	1 (10.0)	
Age, years	66.5 (61.0-72.0)	55.5 (51.0-65.0)	<.01
BMI, kg/m ²	26.7 (23.2-29.2)	25.8 (21.4-26.7)	NS
Maximum resting ABI	0.64 (0.52-0.77)	1.19 (1.13-1.29)	<.0001
Minimum stress ABI	0.38 (0.25-0.77)	e	
PAD-associated risk factors			
Hypertension	24 (60.0)	2 (20.0)	.035
Dyslipidemia	32 (80.0)	4 (40.0)	.020
Active statin use	35 (87.5)	4 (40.0)	.004
Smoking history	39 (97.5)	0 (0.0)	<.001
Type II diabetes	11 (27.5)	0 (0.0)	.092
Coronary artery disease	12 (30.0)	1 (10.0)	NS
Obesity	5 (12.5)	0 (0.0)	NS
Family history	17 (42.5)	5 (50.0)	NS
Chronic kidney disease	2 (5.0)	0 (0.0)	NS
Occlusive disease pattern			
Aortoiliac	6 (15.0)	e	e
Femoropopliteal	7 (17.5)	e	e
Aortoiliac þ femoropopliteal	19 (47.5)	e	e
Femoropopliteal þ tibial	2 (5.0)	e	e
Aortoiliac þ femoropopliteal þ tibial	6 (15.0)	e	e

ABI, Ankle-brachial index; BMI, body mass index; NS, not significant ($P > .05$); PAD, peripheral artery disease.

All continuous variables are expressed as median and interquartile range (IQR); other values are number (%). Comparisons were considered statistically significant at $P < .05$.

Statistical analysis.

The symptomatic extremity that stopped the patient from walking and produced PWT was used for analysis of resting and stress ABI, NIRS measurements, and CTA description in patients with PAD. In patients with PAD with equal, bilateral lower extremity symptoms and in all control participants, the extremity with the lower resting ABI was analyzed.

Data were processed in Microsoft Excel (Microsoft, Redmond, Wash). Statistical analysis was performed by a dedicated statistician using SAS software version 9.4 (SAS Institute Inc., Cary, NC). Post hoc, patients with PAD were separated into low, middle, and high walking tertiles by PWT for comparison. Further, a subanalysis of patients with unilateral abnormalities in resting ABI was performed. In this cohort, the extremity with an ABI of less than 0.9 was compared with the extremity with an ABI of greater than 0.9. Categorical variables were analyzed by Fischer exact and c^2 tests as appropriate. Continuous variables were compared by Wilcoxon rank-sum and

Kruskal-Wallis tests. Correlations between continuous variables, adjusted for the five occlusive disease patterns, were calculated using partial Spearman correlation coefficients. In the tertile comparison all P values were Bonferroni corrected to adjust for multiple comparisons. A P values of less than .05 was considered statistically significant.

RESULTS

Clinical characteristics.

Demographics and clinical characteristics are summarized in [Table I](#). We recruited 40 claudicating patients and 10 control participants. All patients and participants were male and most were Caucasian, reflective of the Nebraska-Western Iowa Veterans Affairs Medical Center patient population. Control participants had higher ABIs, were younger, and had fewer PAD-associated risk factors. Specifically, the incidence of hypertension, dyslipidemia, statin use, and smoking history was significantly lower in the control group ($P < .05$ for all). All patients with PAD completed a CTA with distal runoff. The most common location for occlusive disease was combined aortoiliac and femoropopliteal, followed by isolated femoropopliteal and isolated aortoiliac disease. Eight patients had tibial disease. Six of the eight participants with tibial disease had a single affected runoff vessel and the other two had two- and three-vessel tibial occlusive disease.

PAD versus control NIRS profile comparison.

PAD and control participants completed the Gardner protocol as described. Representative StO_2 profiles of PAD and control calf muscle during the Gardner protocol are presented in [Fig 2](#). Exercise data are presented in [Table II](#). Among the patients with PAD the median COT occurred at 108 seconds and the median PWT at 288 seconds. Baseline StO_2 measurements were similar between the control and PAD cohorts. With initiation of exercise, patients with PAD experienced desaturation below baseline StO_2 (BBT) earlier in their exercise interval as compared with controls ($P < .0001$). For four patients with PAD, the wireless signal was lost temporarily at the time of the start of exercise, preventing the determination of a precise time of desaturation below baseline. These four patients with PAD were not included in the analysis of BBT. For those patients with PAD who were able to walk longer than 540 seconds, data analysis was limited to the first 540 seconds of exercise. Patients with PAD experienced a greater percent decline in StO_2 from baseline at 1 minute and a lower exercise minimum StO_2 compared with controls ($P < .001$ for all measures).

Calf muscle StO_2 profiles of PWT tertiles.

We tested the ability of NIRS parameters to differentiate walking abilities of patients with PAD grouped into tertiles of PWT. Tertile demographics and exercise results are presented in [Tables III](#) and [IV](#). There were no significant differences in demographics, lower extremity hemodynamics (resting and stress ABI), or disease pattern (CTA) among the tertiles. Patient walking time was highest in tertile 1 (T_1 ; $n = 13$) and decreased progressively across tertile 2 (T_2 ; $n = 13$) and tertile 3 (T_3 ; $n = 14$). After the start of exercise, calf muscle StO_2 of the T_1 patients dropped below baseline later than that of T_2 or T_3 patients ($P < .001$). The reduction of StO_2 in T_1 patients was less than that of T_2 and T_3 patients at 1 minute of exercise, COT, PWT, and at the exercise minimum. Additionally, T_1 patients reached the minimum StO_2 later than T_2 and T_3 patients ($P <$

.001). Patients in T₂ compared with those in T₃ experienced a significantly smaller degree of desaturation at 1 minute of exercise and COT.

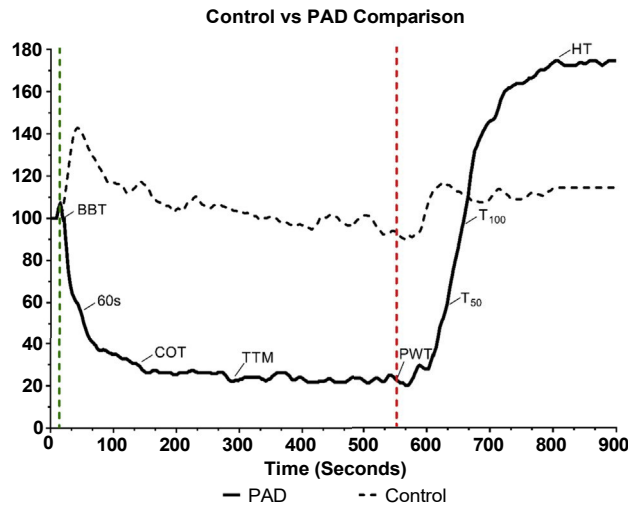


Fig 2. Representative comparison of calf muscle oxygen saturation (StO_2) in control and peripheral artery disease (PAD) patients during exercise and recovery with designation of exercise variables. With standing from the seated baseline position both PAD and control participants experienced an initial increase in StO_2 . With the initiation of exercise patients with PAD rapidly dropped below baseline to a minimum StO_2 . Control participants experienced a gradual decrease in StO_2 throughout the duration of the exercise interval. All StO_2 are expressed as percent of baseline StO_2 for normalization. PAD is designated by the *solid line* and control by the *dotted line*. The *green dotted line*, Initiation of exercise; the *red dotted line*, exercise stop. 60s, StO_2 at 1 minute of exercise; BBT, time to first StO_2 below baseline; COT, claudication onset time; HT, time to maximum StO_2 during recovery interval; PWT, peak walking time; T_{50} , time to 50% recovery of baseline StO_2 ; T_{100} , time to 100% recovery of baseline StO_2 ; TTM, time to minimum StO_2 .

Correlations of walking limitation, hemodynamics, and exercise variables.

We explored the correlations between resting ABI, stress ABI, NIRS measurements, and Gardner variables and the two measures of walking ability 6MWD and PWT (Table V). Resting ABI did not correlate with the 6MWD ($r_{1/4}$ 0.17; $P_{1/4}$.33) or PWT ($r_{1/4}$ 0.12; $P_{1/4}$.48; Fig 3). Minimum stress ABI was modestly correlated with PWT ($r_{1/4}$ 0.33; $P_{1/4}$.046) but not 6MWD. 6MWD was strongly correlated with percent StO_2 change at 1 minute of exercise, COT, PWT, and time to minimum StO_2 ($r_{1/4}$ -0.67, 0.44, 0.58, and 0.70, respectively; $P < .05$ for all). PWT was strongly correlated with percent change in StO_2 at 1 minute of exercise ($r_{1/4}$ -0.76; $P < .001$; Fig 4), at COT (-0.75; $P < .001$), at PWT (-0.59; $P < .0001$), at exercise minimum (-0.61; $P < .001$), and with time to minimum StO_2 (0.79; $P < .0001$). During the recovery phase, T_{50} and T_{100} had a moderate correlation with PWT ($r_{1/4}$ -0.53 and -0.53 respectively; $P < .001$ for both) but not with 6MWD ($r_{1/4}$ -0.01 and -0.03 respectively; $P > .05$ for both).

Comparison of extremities in patients with PAD with unilateral abnormalities in resting ABI.

Within the total cohort of 40 patients with PAD, 8 participants (20.0%) had unilateral abnormalities in resting ABI (Table VI). One of the eight extremities with abnormal resting ABI had isolated aortoiliac disease, one had isolated femoropopliteal disease, five had combined aortoiliac and femoropopliteal disease, and one had combined aortoiliac, femoropopliteal, and tibial disease. In the eight extremities with a normal resting ABI, we identified appreciable atherosclerotic disease in five extremities (62.5%); three extremities (37.5%) had no radiographic evidence of occlusive disease. One of the five extremities with appreciable disease had isolated mild aortoiliac disease, one had isolated moderate aortoiliac disease, two had mild aortoiliac and mild to moderate femoropopliteal disease, and one had mild aortoiliac and mild tibial disease. All extremities with an ABI of greater than 0.9 had compressible pedal arteries and demonstrated three-vessel runoff by CTA. The median resting ABI was higher in the extremity with normal ABI as compared with the extremity with abnormal ABI (1.02; [interquartile range {IQR}, 0.92-1.00] vs 0.73 [IQR, 0.65-0.78], respectively; $P < .001$). Similarly, the median stress ABI was higher in the extremity with normal resting ABI when compared with the abnormal extremity (0.84 [IQR, 0.80- 0.90] vs 0.62 [IQR, 0.45-0.63], respectively; $P < .01$). Median PWT in this cohort was 505 seconds (IQR, 418-650). During treadmill exercise, all eight extremities with an abnormal ABI became symptomatic, whereas only three of the eight extremities with a normal ABI developed symptoms of IC. Calf muscle StO₂ decreased significantly more at 1 minute of exercise, at exercise minimum, and at PWT in the extremity with abnormal ABI as compared with the extremity with normal ABI ($P < .05$ for all). The T₅₀ occurred sooner in extremities with normal resting ABI ($P < .05$). All other recovery variables were similar.

Table II. Comparison of peripheral artery disease (PAD) and control exercise variables

	PAD	Control	P value
Total number	40	10	e
Baseline StO ₂	50 (40-53)	54 (43-68)	NS
Six-minute walking distance, meters	281 (210-335)	e	e
Exercise variables			
BBT, seconds	1 (0-16)	104 (61-162)	<.0001
Absolute StO ₂ change at 1 minute	-31 (-44 to -16)	4 (-1 to 9)	<.0001
Percent StO ₂ change 1 minute, %	-73.2 (-90.6 to -36.2)	8.3 (-18.6 to 2.4)	<.0001
COT, seconds	108 (69-179)	e	e
Absolute StO ₂ change at COT	-31 (-42 to -17)	e	e
Percent StO ₂ Change at COT, %	-69.1 (-89.5 to -40.2)	e	e
PWT, seconds	288 (180-529)	e	e
Absolute StO ₂ change at PWT	-35 (-44 to -25)	e	e
Percent StO ₂ change at PWT, %	-74.6 (-92.8 to -57.2)	e	e
Time to Minimum StO ₂ , seconds	119 (60-399)	522 (505-532)	<.001
Absolute StO ₂ change at minimum	-37 (-47 to -26)	-11 (-18 to -3)	<.0001
Percent StO ₂ change at minimum, %	-83.4 (-97.6 to -62.4)	-16.1 (-32.3 to -7.8)	<.0001

BBT, Below baseline time; COT, claudication onset time; IQR, interquartile range; NS, ($P > .05$); PWT, peak walking time; StO₂, oxygen saturation. All continuous variables are expressed as median and interquartile range (IQR). Comparisons were considered statistically significant with $P < .05$.

The eight extremities with a normal resting ABI in patients with PAD were also compared with control extremities. The resting ABI in control extremities was higher than the resting ABI in the extremities with normal ABI in patients with PAD (1.19 [IQR, 1.13-1.29] vs 1.02 [IQR, 0.92-1.00],

respectively; $P = .028$). Resting StO_2 was higher in the control extremities as compared with normal ABI extremities in patients with PAD; however, this difference was not significant (54 [IQR, 44-68] vs 40 [IQR, 34-53], respectively; $P = .33$). Normal ABI extremities in patients with PAD, as compared with controls, had a greater percentage change in StO_2 at 1 minute of exercise (-9.6% [IQR, -28.3% to 1.5%] vs 8.3% [IQR, -18.6% to 2.4%], respectively; $P < .05$) and at exercise minimum (-59.1% [IQR, -64.7% to -38.2%] vs -16.1% [IQR, -32.3% to -7.8%], respectively; $P = .012$), with similar median times to minimum StO_2 (483 seconds [IQR, 210-590 seconds] vs 522 seconds [IQR, 505-532 seconds]; $P = .26$).

DISCUSSION

In this study, we report the use of a low-cost, wireless NIRS device during a standardized treadmill evaluation in claudicating participants with PAD and in healthy controls. Lower extremities affected by PAD exhibited distinct calf muscle NIRS profiles during exercise characterized by a rapid decrease in StO_2 , substantial hypoxia throughout exercise, and slowed recovery to baseline StO_2 after exercise. In patients with a contralateral extremity with normal resting ABI, the NIRS findings characteristic of PAD were present when atherosclerotic narrowing of the major arteries was documented by CTA. Further, we found the degree of calf muscle hypoxia experienced during exercise strongly and inversely correlated with walking capacity, measured by PWT and 6MWD. Taken together, this work suggests NIRS captures the pathologic state of muscle oxygen balance in PAD and provides a window into the complex nature of PAD-induced walking impairment.

We believe that the profoundly altered StO_2 profiles and severe walking limitation of patients with IC is a product of multiple pathologic features of PAD. PAD starts as atherosclerotic disease affecting the large arteries supplying the leg (macrovascular disease) and this produces substantial limitation of the blood flow to the claudicating extremity. In addition to the macrovascular blood flow restriction, work from our group and others suggests that patients with PAD exhibit “microvascular disease” seen as a thickening of capillary basement membranes, perivascular fibrosis, and impaired endothelial reactivity.¹⁶⁻¹⁹ This microvascular disease further impairs the delivery of oxygen and nutrients to the affected tissues. Moreover, studies of permeabilized myo- fibers have demonstrated a significant dysfunction in the mitochondria of affected muscle cells, decreasing the ability of the muscle to effectively use the delivered oxygen and nutrients.²⁰⁻²⁴ In addition to these limitations in oxygen delivery and use, the skeletal muscle of patients with PAD suffers from a chronic, ischemic myopathy that further impairs its ability to work. This myopathy is characterized by irregular myofiber morphology, cytoskeletal disruption, oxidative damage to myofibers and blood vessels, and fibrosis.^{14,24-29} It is the combination of these pathologic features of PAD that collaborates with macrovascular disease to produce the profoundly altered StO_2 profiles and severe walking limitation of claudicating patients.

Table III. Peak walking time (PWT) tertile demographics

	Tertile 1 (>463 seconds)	Tertile 2 (241-463 seconds)	Tertile 3 (<241 seconds)
Total number	13	13	14
Race			
Caucasian	12 (92.3)	12 (92.3)	14 (100.0)
African American	1 (7.7)	1 (7.7)	0 (0.0)
Age, years	65 (60-68)	67 (65-72)	69 (60-73)
BMI, kg/m ²	27.3 (24.7-31.9)	27.1 (23.3-32.2)	24.4 (22.1-28.6)
Maximum resting ABI	0.65 (0.52-0.77)	0.69 (0.59-0.83)	0.63 (0.41-0.74)
Minimum stress ABI	0.39 (0.27-0.62)	0.47 (0.33-0.52)	0.28 (0.20-0.42)
PAD-associated risk factors			
Hypertension	8 (61.5)	9 (69.2)	7 (50.0)
Dyslipidemia	11 (92.3)	10 (76.9)	10 (71.4)
Active statin use	4 (30.8)	4 (30.8)	3 (21.4)
Smoking history	13 (100.0)	12 (92.3)	14 (100.0)
Type II diabetes	12 (92.3)	10 (100.0)	11 (78.6)
Coronary artery disease	5 (38.5)	5 (38.5)	2 (14.3)
Obesity	2 (15.4)	2 (15.4)	1 (7.1)
Family history	6 (46.2)	6 (46.2)	5 (35.7)
Chronic kidney disease	1 (7.7)	1 (7.7)	0 (0.0)
Occlusive disease pattern			
Aortoiliac	2 (15.3)	2 (15.3)	2 (14.3)
Femoropopliteal	1 (7.7)	4 (30.8)	2 (14.3)
Aortoiliac \bar{p} femoropopliteal	9 (69.3)	4 (30.8)	6 (42.9)
Femoropopliteal \bar{p} tibial	1 (7.7)	1 (7.7)	0 (0.0)
Aortoiliac \bar{p} femoropopliteal \bar{p} tibial	0 (0.0)	2 (15.3)	4 (28.5)

ABI, Ankle-brachial index; BMI, body mass index; PAD, peripheral artery disease.

There were no significant differences in the tertile demographics or PAD-associated risk factors. Occlusive disease patterns were statistically similar. All continuous variables are expressed as median and interquartile range (IQR); other values are number (%). Comparisons were considered statistically significant at $P < .05$.

Our work demonstrates that an easily applied, inexpensive, wireless NIRS monitor can generate similar calf muscle StO₂ profiles as compared with those previously documented with the use of costly, wired devices.^{10,11} Gardner et al¹¹ reported calf muscle StO₂ decreased 74% at exercise minimum in a cohort of 39 patients with PAD and IC. The authors additionally found that time to minimum StO₂ ($r = 0.68$; $P < .001$), recovery half-time of StO₂ after exercise ($r = -0.49$; $P < .01$), and recovery time of StO₂ after exercise ($r = -0.48$; $P < .01$) were all correlated with PWT during a standardized treadmill protocol.¹¹ Similarly, Afaq et al³⁰ have reported calf muscle StO₂ at 1 minute of exercise was moderately correlated with PWT ($r = 0.44$; $P < .01$).³⁰ In our study, patients with PAD experienced a similar mean desaturation at exercise minimum (79.8%). In addition, the device used in our work produced strong correlations of StO₂ change at 1 minute of exercise, TTM, T₅₀, and T₁₀₀ to PWT, similar to previous studies.

This finding is important, because the cost and physical limitations imposed by wired NIRS devices may, in part, explain their limited adoption outside well-funded research laboratories. Contemporary wired NIRS systems exceed US\$30,000 to US\$40,000 for a single setup, and many traditionally used NIRS devices are no longer commercially available. Similarly, modern wireless NIRS systems cost in excess of US\$9,000. In contrast, the NIRS probe used in this study is available for US\$819. Moreover, the lightweight, wireless, and low-profile design of the device precludes any influence a wired component may have on the participants' gait or degree of walking impairment.

In our study, healthy control extremities had minimal change in StO₂ at 1 minute of exercise. In contrast, extremities affected by PAD demonstrated sharp decreases in resting StO₂ early in exercise, and this desaturation was inversely correlated with walking ability. These findings may allow for earlier diagnosis of patients with PAD and characterization of disease severity. Future work

should focus on generating clinically relevant NIRS profiles; one can envision NIRS-based devices not only used in clinic to detect PAD, but also as a tool for vascular surgeons to estimate risk of functional decline and possibly predict and monitor response to available therapies.

Table IV. Peak walking time (PWT) tertile exercise and recovery data

	Tertile 1 (PWT >463 seconds)	Tertile 2 (PWT 241-463 seconds)	Tertile 3 (PWT <241 seconds)
Total number	13	13	14
Baseline StO ₂	50 (41-54)	50 (39-52)	46 (43-53)
Six-minute walking distance, meters	345 (293-422)	283.5 (223-314)	237 (158.5-268) ^a
Exercise variables			
BBT, seconds	22 (1-34)	1 (0-5) ^a	1 (0-3) ^a
Absolute StO ₂ change at 1 minute	-7 (-18 to 1)	-32 (-40 to -26) ^a	-44 (-50 to -35) ^b
Percent StO ₂ change 1 minute, %	-20.0 (-41.5 to 1.8)	-76.1 (-90.0 to -49.2) ^a	-90.6 (-79.3 to -100) ^b
COT, seconds	230 (131-271)	109 (88-161) ^b	66 (55-102) ^{b,c}
Absolute StO ₂ change at COT	-11 (-19 to -8)	-32 (-39 to -25) ^b	-42 (-48 to -34) ^b
Percent StO ₂ change at COT, %	-25.7 (-38.0 to -19.0)	-78.1 (-89.1 to -61.1) ^b	-89.4 (-100.0 to -79.6) ^b
PWT, seconds	611 (529-840)	291 (272-306)	164.5 (129-185)
Absolute StO ₂ change at PWT	-26 (-32 to -19)	-37 (-25 to -42)	-43 (-47 to -33) ^b
Percent StO ₂ change at PWT	-57.4 (-70.7 to -46.3)	-84.1 (-84.1 to -56.7)	-92.1 (-99.3 to -75.3) ^b
Time to minimum StO ₂ , seconds	544 (476-791)	104 (37-270)	69 (36-77)
Absolute StO ₂ change at minimum	-31 (-34 to -22)	-39 (-47 to -31) ^a	-44 (-51 to v38) ^b
Percent StO ₂ change at minimum	-69.1 (-75.9 to -46.4)	-90.2 (-100.0 to -72.2) ^b	-95.1 (-100.0 to -88.2) ^b
Recovery slope, StO ₂ /second	0.25 (0.15-0.49)	0.20 (0.11-0.32)	0.14 (0.07-0.24)
T ₅₀ , seconds	65 (43-123)	104 (50-126)	196 (120-304)
T ₁₀₀ , seconds	103 (69-165)	160 (83-318)	289 (183-435)
Time to recovery maximum StO ₂ , seconds	372 (243-593)	282 (148-1106)	469 (315-725)
Absolute StO ₂ change at recovery maximum	19 (16-25)	26 (13-37)	20 (1-29)
Percent StO ₂ change at recovery maximum	37.7 (29.6-48.1)	72.4 (17.3-85.2)	37.0 (3.3-70.3)

BBT, Below baseline time; COT, claudication onset time; StO₂, oxygen saturation; T₅₀, time to 50% recovery of baseline StO₂; T₁₀₀, time to 100% recovery of baseline StO₂.

All continuous variables are expressed as median and interquartile range (IQR). Comparisons were considered statistically significant at $P < .05$. Comparison to T₁: ^a $P < .05$, ^b $P < .01$.

Comparison of T₂ to T₃: ^c $P < .05$.

In our study, the calf muscle StO₂ of patients with PAD decreased below the baseline significantly earlier during exercise as compared with controls. Using synchronized data from our instrumented treadmill device, we found that patients with PAD on average dropped below baseline StO₂ at 12 steps (range, 1-55 steps). This finding suggests that the calf muscles of claudicating patients may become ischemic well before any symptoms occur and even when walking surprisingly short distances.

There is debate regarding the optimal method to quantify the degree of walking impairment in patients with PAD. Authors have argued in favor of either treadmill-based measurements or over ground, timed walking tests.^{31,32} Although treadmill-based examination explores the patient's physiologic maximum and the 6-minute walking test better emulates daily over-ground walking, our NIRS data suggests the tests share a common physiologic underpinning. We found that PWT correlated well with the 6MWD ($r = 0.58$; $P < .001$) and that NIRS profiles in participants with PAD were highly correlated with both metrics of walking performance. Percent StO₂ change at 1 minute of exercise and the time to minimum StO₂ strongly correlated with the MWD ($r = -0.67$ and 0.70 , respectively; $P < .0001$ for both). Similarly, as others have demonstrated, percent StO₂ change at 1 minute of exercise and time

to minimum StO₂ have an even stronger correlation to PWT ($r = -0.76$ and -0.79 , respectively; $P < .0001$ for both).³⁰ Our data clearly demonstrate that the combination of time frame and the degree calf muscle ischemia seem to be a powerful correlate of walking capacity.

Limitations.

The overall contribution of intracellular myoglobin in the determination of StO₂ is challenging to quantify. However, its use as a measure of local tissue oxygen extraction is well accepted. Koga et al³⁰ using an in vitro model of skeletal muscle contraction in rat gastrocnemius compared deoxygenation kinetics of visible light spectroscopy and phosphorescence-quenching measures of PO₂ (a surrogate for pure hemoglobin deoxygenation).³³ The authors demonstrated similar desaturation time constants in both measures; however, the time delay to initial desaturation was prolonged in visible light spectroscopy. Importantly, the mean response time (time delay + time constant) was not significantly different between the measures. This finding suggests StO₂ is an appropriate surrogate of local oxygen availability and, therefore, useful in understanding the changes in the calf muscle oxygenation during exercise.³³

Baker et al³⁴ have reported enhanced maximal calf muscle blood flow and oxygen extraction after 3 months of exercise therapy in patients with PAD, using a device that combined the capability of frequency domain NIRS with that of diffuse correlation spectroscopy. However, these findings were not reflected in resting measurements of blood flow or resting muscle StO₂.³⁴ Similarly, Beckitt et al³⁵ studied patients with PAD receiving exercise therapy or successful angioplasty and did not report changes in resting gastrocnemius StO₂, but found significant decrease in calf muscle desaturation at a submaximal exercise time point of 100 seconds in both the exercise and angioplasty cohorts. Furthermore, in a limited study of a single patient treated by angioplasty of iliac stenosis, Henni et al³⁶ showed that transcutaneous oxygen measures obtained during exercise testing are normalized after revascularization. Overall the improvements seen in calf muscle StO₂ during exercise would suggest that a short duration treadmill evaluation with concurrent NIRS measurements may be a more precise and comprehensive alternative to resting NIRS measurements for documenting treatment response. Given the strong correlation of walking performance and changes in gastrocnemius StO₂ early in exercise, we would put forth the idea of a 1-minute treadmill evaluation with concurrent NIRS to monitor patients during treatment. In our experience, this protocol can be completed within 15 minutes and could reasonably be performed at the time of a clinic visit. We anticipate that therapies that improve, leg hemodynamics, muscle perfusion, oxygen balance, and/or ischemic myopathy would show improvements in NIRS profiles as has been shown by Baker et al,³⁴ Beckitt et al,³⁵ and Henni et al.³⁶ However, it is not likely that NIRS devices have the capacity to decipher the precise mechanisms (improved hemodynamics vs improved myopathy, etc) behind the documented enhancements of the StO₂ and additional testing of muscle perfusion, biochemistry and histology should be included to address this type of more complicated mechanistic questions.

Table V. Correlations of exercise and recovery variables to measures of walking impairment

	Six-minute walking distance	PWT
Maximum resting ABI	0.17	0.12
Minimum stress ABI	0.26	0.33 ^a
Absolute StO ₂ change at 1 minute	-0.67 ^b	-0.72 ^b
Percent StO ₂ change at 1 minute	-0.67 ^b	-0.76 ^b
COT	0.44 ^a	0.70 ^b
Absolute StO ₂ change at COT	-0.55 ^b	-0.70 ^b
Percent StO ₂ change at COT	-0.57 ^b	-0.75 ^b
PWT	0.58 ^b	e
Absolute StO ₂ change at PWT	-0.31	-0.51 ^b
Percent StO ₂ change at PWT	-0.34	-0.59 ^b
Time to minimum StO ₂	0.70 ^b	0.79 ^b
Absolute StO ₂ change at exercise minimum	-0.36 ^a	-0.46 ^c
Percent StO ₂ change at exercise minimum	-0.43 ^a	-0.61 ^b
Time to 50% recovery	-0.23	-0.53 ^b
Time to 100% recovery	-0.29	-0.52 ^b
Time to recovery maximum StO ₂	-0.08	0.03
Absolute StO ₂ change at recovery maximum	0.01	0.19
Percent StO ₂ change at recovery maximum	0.03	0.19

COT, Claudication onset time; PWT, peak walking time; StO₂, oxygen saturation.

^aP < .05.

^bP < .001.

^cP < .01.

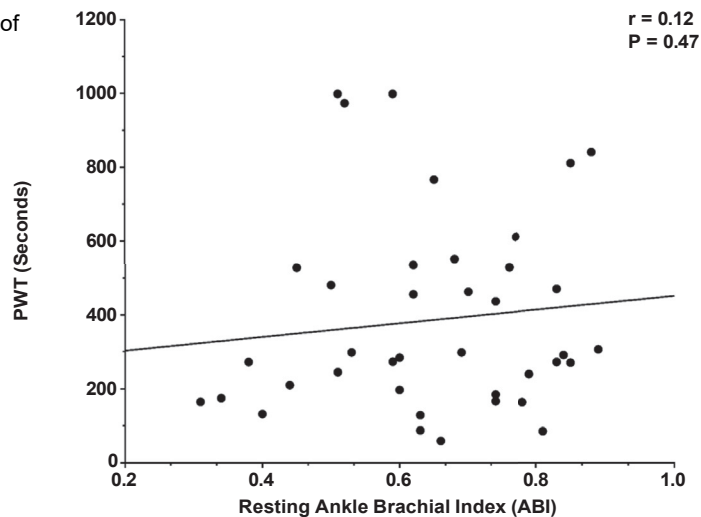


Fig 3. Correlation of resting ankle-brachial index (ABI) with peak walking time (PWT). Partial Spearman correlation coefficient adjusted for occlusive disease pattern.

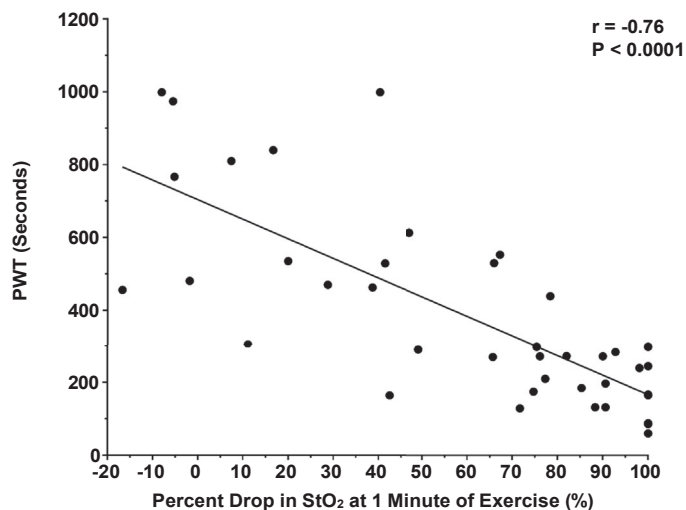


Fig 4. Correlation of percent decrease in oxygen saturation (StO₂) at 1 minute of exercise and peak walking time (PWT). Partial Spearman correlation coefficient adjusted for occlusive disease pattern.

Important differences between the control and PAD cohort warrant discussion. Patients with PAD had significantly higher rates of smoking as compared with controls. It has been shown previously that, after adjusting for maximal blood flow, patients who are active smoking and have PAD experience lower calf muscle StO₂ during exercise than patients who do not smoke and have PAD.³⁰ Because none of our control patients smoked, this represents a possible confounder in the significantly lower StO₂ measurements found in the PAD cohort at all points in exercise. Similarly, microvascular responsiveness is decreased with aging and may contribute to the lower StO₂ seen in the PAD cohort.³⁷ Control participants did not undergo a stress ABI measurement. It is, therefore, possible control participants with clinically asymptomatic PAD entered the study. If any clinically silent patients with PAD were recruited as controls, it is not obvious from the analysis. In addition,

our study did not enroll any women and the PAD cohort was largely Caucasian, limiting the generalizability of the results.

Last, there are limitations in using a treadmill walking test as an indicator of walking impairment. Patients unable to participate in the full treadmill protocol were excluded. The results of this study are not generalizable to patients with decreased walking velocity or severe walking impairment.³¹ However, for the purpose of comparing differences in calf muscle NIRS profiles, a standardized work load is optimal.

Table VI. Comparison of normal resting ankle-brachial index (*ABI*) extremities with abnormal resting *ABI* extremities in patients with unilateral abnormalities in resting *ABI*

	Normal resting ABI extremity (n ¼ 8)	Abnormal resting ABI extremity (n ¼ 8)	P value
Hemodynamic variables			
CTA presence of atherosclerosis	5 (62.5)	8 (100.0)	NS
Maximum resting ABI	1.02 (0.92-1.00)	0.73 (0.65-0.78)	<.001
Minimum stress ABI	0.84 (0.80-0.90)	0.62 (0.45-0.63)	<.01
Exercise variables			
Baseline StO ₂	40 (34-53)	42 (39-53)	NS
Absolute StO ₂ change at 1 minute	-5 (-9 to 1)	-16 (-21 to -5)	<.01
Percent StO ₂ change at 1 minute	-9.6 (-28.3 to 1.5)	-40.1 (-50.8 to -12.1)	<.01
Absolute StO ₂ change at PWT	-18 (-23 to -12)	-27 (-33 to -23)	NS
Percent StO ₂ change at PWT	-45.1 (-58.2 to -33.2)	-68.3 (-78.5 to -46.4)	.03
Time to minimum StO ₂ , seconds	483 (210-590)	503 (278-554)	NS
Absolute StO ₂ change at minimum	-21 (-23 to -20)	-29 (-34 to -25)	.054
Percent StO ₂ change at minimum	-59.1 (-64.7 to -38.2)	-74.0 (-80.2 to -56.4)	.020
Recovery variables			
Recovery slope, StO ₂ /second	0.32 (0.25-0.38)	0.19 (0.14-0.36)	NS
T ₅₀ , seconds	24 (10-47)	77 (41-153)	.039
T ₁₀₀ , seconds	55 (37-152)	148 (73-203)	NS
Time to recovery maximum StO ₂ , seconds	365 (127-1420)	320 (229-673)	NS
Absolute StO ₂ change at recovery maximum	24 (16-30)	24 (12-34)	NS
Percent StO ₂ change at recovery maximum	66.5 (32.1-83.3)	56.4 (25.5-75.6)	NS

CTA, Computed tomographic angiography; NS, not significant; PWT, peak walking time; StO₂, oxygen saturation; T₅₀, time to 50% recovery of baseline StO₂; T₁₀₀, time to 100% recovery of baseline StO₂.

Comparisons were considered statistically significant at $P < .05$. Values are median (interquartile range [IQR]) or number (%).

CONCLUSIONS

In this initial evaluation of a novel, low-cost NIRS device, lower extremities affected by PAD exhibited characteristic changes in calf muscle StO₂ that differentiated them from healthy controls. In agreement with previous studies using traditional NIRS devices, the degree of walking impairment as measured by PWT and 6MWD was strongly correlated with changes in StO₂ early in exercise across a variety of occlusive disease patterns and degrees of hemodynamic impairment. These data suggest NIRS may be a feasible method to distinguish between arterial-related walking impairment and walking impairment owing to nonarterial causes. Additionally, as measured by NIRS, the ability to maintain muscle oxygenation is an important driver of walking capacity in PAD. Further research should investigate the ability of low-cost, wireless NIRS technology to evaluate, diagnose, and monitor treatment response in PAD.

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AUTHOR CONTRIBUTIONS

Conception and design: MF, HH, HY, SM, GC, IP Analysis and interpretation: MF, HH, YG, MS, SM, GC, IP Data collection: MF, HH, YG, HY, MS, IP

Writing the article: MF, HH, YG, HY, MS, SM, GC, IP Critical revision of the article: MF, HH, GC, IP

Final approval of the article: MF, HH, YG, HY, MS, SM, GC, IP

Statistical analysis: MF, GC, IP Obtained funding: Not applicable Overall responsibility: MF

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REFERENCES

1. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, et al. Heart disease and stroke statistics- 2018 update: a report from the American Heart Association. *Circulation* 2018;137:e67-492.
2. Fowkes FG, Rudan D, Rudan I, Aboyans V, Denenberg JO, McDermott MM, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet* 2013;382:1329-40.
3. McDermott MM, Liu K, Greenland P, Guralnik JM, Criqui MH, Chan C, et al. Functional decline in peripheral arterial disease: associations with the ankle brachial index and leg symptoms. *JAMA* 2004;292:453-61.
4. McDermott MM, Greenland P, Liu K, Guralnik JM, Criqui MH, Dolan NC, et al. Leg symptoms in peripheral arterial disease: associated clinical characteristics and functional impairment. *JAMA* 2001;286:1599-606.
5. Hernandez H, Myers SA, Schieber M, Ha DM, Baker S, Koutakis P, et al. Quantification of daily physical activity and sedentary behavior of claudicating patients. *Ann Vasc Surg* 2019;55:112-21.
6. Regensteiner JG, Hiatt WR, Coll JR, Criqui MH, Treacher J, Jacobson D, McDermott MM, et al. The impact of peripheral arterial disease on health-related quality of life in the Peripheral Arterial Disease Awareness, Risk, and Treatment: New Resources for Survival (PARTNERS) Program. *Vasc Med* 2008;13:15-24.
7. Ankle Brachial Index Collaboration, Fowkes FG, Murray GD, Butcher I, Heald CL, Lee RJ, et al. Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: a meta-analysis. *JAMA* 2008;300:197-208.
8. McDermott MM, Mehta S, Greenland P. Exertional leg symptoms other than intermittent claudication are common in peripheral arterial disease. *Arch Intern Med* 1999;159: 387-92.
9. McDermott MM, Ferrucci L, Liu K, Guralnik JM, Tian L, Liao Y, et al. Leg symptom categories and rates of mobility decline in peripheral arterial disease. *J Am Geriatr Soc* 2010;58: 1256-62.
10. Comerota AJ, Throm RC, Kelly P, Jaff M. Tissue (muscle) oxygen saturation (StO₂): a new measure of symptomatic lower-extremity arterial disease. *J Vasc Surg* 2003;38:724-9.
11. Gardner AW, Parker DE, Webb N, Montgomery PS, Scott KJ, Blevins SM. Calf muscle hemoglobin oxygen saturation characteristics and exercise performance in patients with intermittent claudication. *J Vasc Surg* 2008;48:644-9.
12. Guyatt GH, Sullivan MJ, Thompson PJ, Fallen EL, Pugsley SO, Taylor DW, et al. The 6-minute walk: a new measure of exercise capacity in patients with chronic heart failure. *Can Med Assoc J* 1985;132:919-23.
13. Schmitz R. U.S. Patent No. 8,941,830. Washington, DC: U.S. Patent and Trademark Office; 2015.
14. McManus CJ, Collison J, Cooper CE. Performance comparison of the MOXY and PortaMon near-infrared spectroscopy muscle oximeters at rest and during exercise. *J Biomed Opt* 2018;23:1-14.

15. Thiel C, Vogt L, Himmelreich H, Hubscher M, Banzer W. Reproducibility of muscle oxygen saturation. *Int J Sports Med* 2011;32:277-80.
16. Koutakis P, Miserlis D, Myers SA, Kim JK, Zhu Z, Papoutsi E, et al. Abnormal accumulation of desmin in gastrocnemius myofibers of patients with peripheral artery disease: associations with altered myofiber morphology and density, mitochondrial dysfunction and impaired limb function. *J Histochem Cytochem* 2015;63:256-69.
17. Ha DM, Carpenter LC, Koutakis P, Swanson SA, Zhu Z, Hanna M, et al. Transforming growth factor-beta 1 produced by vascular smooth muscle cells predicts fibrosis in the gastrocnemius of patients with peripheral artery disease. *J Transl Med* 2016;14:39.
18. Baum O, Djonov V, Ganster M, Widmer M, Baumgartner I. Arteriolization of capillaries and FGF-2 upregulation in skeletal muscles of patients with chronic peripheral arterial disease. *Microcirculation* 2005;12:527-37.
19. Zahner GJ, Spaulding KA, Ramirez JL, Schaller MS, Walker SC, Hills NK, et al. Characterizing the relationship between flow-mediated vasodilation and radial artery tonometry in peripheral artery disease. *J Surg Res* 2018;224:121-31.
20. Pipinos II, Sharov VG, Shepard AD, Anagnostopoulos PV, Katsamouris A, Todor A, et al. Abnormal mitochondrial respiration in skeletal muscle in patients with peripheral arterial disease. *J Vasc Surg* 2003;38:827-32.
21. Makris KI, Nella AA, Zhu Z, Swanson SA, Casale GP, Gutti TL, et al. Mitochondriopathy of peripheral arterial disease. *Vascular* 2007;15:336-43.
22. Pipinos II, Judge AR, Zhu Z, Selsby JT, Swanson SA, Johanning JM, et al. Mitochondrial defects and oxidative damage in patients with peripheral arterial disease. *Free Radic Biol Med* 2006;41:262-9.
23. Pipinos II, Shepard AD, Anagnostopoulos PV, Katsamouris A, Boska MD. Phosphorus 31 nuclear magnetic resonance spectroscopy suggests a mitochondrial defect in claudicating skeletal muscle. *J Vasc Surg* 2000;31:944-52.
24. Pipinos II, Judge AR, Selsby JT, Zhu Z, Swanson SA, Nella AA, et al. The myopathy of peripheral arterial occlusive disease: part 1. Functional and histomorphological changes and evidence for mitochondrial dysfunction. *Vasc Endovascular Surg* 2007;41:481-9.
25. Pipinos II, Judge AR, Selsby JT, Zhu Z, Swanson SA, Nella AA, et al. The myopathy of peripheral arterial occlusive disease: part 2. Oxidative stress, neuropathy, and shift in muscle fiber type. *Vasc Endovascular Surg* 2008;42:101-12.
26. Brass EP, Hiatt WR. Acquired skeletal muscle metabolic myopathy in atherosclerotic peripheral arterial disease. *Vasc Med* 2000;5:55-9.
27. Weiss DJ, Casale GP, Koutakis P, Nella AA, Swanson SA, Zhu Z, et al. Oxidative damage and myofiber degeneration in the gastrocnemius of patients with peripheral arterial disease. *J Transl Med* 2013;11:230.
28. Koutakis P, Myers SA, Cluff K, Ha DM, Haynatzki G, McComb RD, et al. Abnormal myofiber morphology and limb dysfunction in claudication. *J Surg Res* 2015;196: 172-9.
29. Cluff K, Miserlis D, Naganathan GK, Pipinos II, Koutakis P, Samal A, et al. Morphometric analysis of gastrocnemius muscle biopsies from patients with peripheral arterial disease: objective grading of muscle degeneration. *Am J Physiol Regul Integr Comp Physiol* 2013;305:R291-9.
30. Afaq A, Montgomery PS, Scott KJ, Blevins SM, Whitsett TL, Gardner AW. The effect of current cigarette smoking on calf muscle hemoglobin oxygen saturation in patients with intermittent claudication. *Vasc Med* 2007;12:167-73.
31. McDermott MM, Guralnik JM, Criqui MH, Liu K, Kibbe MR, Ferrucci L. Six-minute

- walk is a better outcome measure than treadmill walking tests in therapeutic trials of patients with peripheral artery disease. *Circulation* 2014;130:61-8.
32. Hiatt WR, Rogers RK, Brass EP. The treadmill is a better functional test than the 6-minute walk test in therapeutic trials of patients with peripheral artery disease. *Circulation* 2014;130:69-78.
 33. Koga S, Kano Y, Barstow TJ, Ferreira LF, Ohmae E, Sudo M, et al. Kinetics of muscle deoxygenation and microvascular PO₂ during contractions in rat: comparison of optical spectroscopy and phosphorescence-quenching techniques. *J Appl Physiol* (1985) 2012;112:26-32.
 34. Baker WB, Li Z, Schenkel SS, Chandra M, Busch DR, Englund EK, et al. Effects of exercise training on calf muscle oxygen extraction and blood flow in patients with peripheral artery disease. *J Appl Physiol* (1985) 2017;123:1599-609.
 35. Beckitt TA, Day J, Morgan M, Lamont PM. Calf muscle oxygen saturation and the effects of supervised exercise training for intermittent claudication. *J Vasc Surg* 2012;56: 470-5.
 36. Henni S, Bauer P, Le Meliner T, Hersant J, Papon X, Daligault M, et al. High prevalence of exercise-induced ischemia in the asymptomatic limb of patients with apparently strictly unilateral symptoms and unilateral peripheral artery disease. *Ther Adv Cardiovasc Dis* 2019;13. 1753944718819063.
 37. Seals DR, Jablonski KL, Donato AJ. Aging and vascular endothelial function in humans. *Clin Sci (Lond)* 2011;120:357-75.