ORIGINAL RESEARCH ARTICLE

Calf Tissue Oxygenation During Exercise in Men with and Without Risk Factors for Developing Peripheral Arterial Disease

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ABSTRACT

Miranda A, Figoni SF, Cha T, Flanagan T, Mandal O, Silva M, Scremin AME, Scremin OU: Calf tissue oxygenation during exercise in men with and without risk factors for developing peripheral arterial disease. Am J Phys Med Rehabil 2012;91:200–210.

Objective: This study aimed to compare calf tissue oxygenation responses to calf exercise in men without diagnosed peripheral arterial disease but with selected risk factors for peripheral arterial disease with those without risk factors.

Design: A cross-sectional quasi-experimental design was used. The no-risk group ($n = 20$) had none of the risk factors (diabetes, hypertension, hyperlipidemia, obesity, current or 10 pack-yr smoking history, or age ≥65 yrs). The at-risk group ($n = 45$) had one to six risk factors. Medial calf tissue oxygenation (percentage saturation) was determined using near-infrared spectroscopy during seven consecutive 5-min test stages: rest, 0-W active plantar/dorsiflexion, rest, 4-W resistive plantar flexion, rest, 8-W resistive plantar flexion, and rest. Resistive exercise was performed on the Stresst'er calf ergometer.

Results: Compared with the no-risk group, decrements in calf tissue oxygenation induced by light-to-moderate resistive calf exercise in the at-risk group was significantly greater (by 9% saturation) (4-W: $P < 0.001$; 8-W: $P = 0.002$).

Conclusions: Men with risk factors for developing peripheral arterial disease but without such diagnosis demonstrated greater decrements in calf tissue oxygenation during calf exercise compared with men without risk factors. Further development of this test may lead to early diagnosis and intervention to modify risk factors and prevent co-morbidities.

Key Words: Rehabilitation, Exercise Test, Spectroscopy, Near-Infrared, Peripheral Vascular Diseases

Peripheral arterial disease (PAD) refers to atherosclerotic occlusive disease of the arterial system distal to the aortic bifurcation. One of the most common symptoms of PAD is intermittent claudication, defined as fatigue, discomfort, or pain in lower-limb muscle groups resulting from exercise-induced ischemia usually relieved by rest.[1] Total mortality after PAD diagnosis is 11% after 2 yrs and 65% after an average of 6.6 yrs.[2] Other comorbidities include lower-limb ischemia resulting in skin ulceration, infection, and amputation. The prevalence of PAD based on ankle-brachial index testing is approximately 10%–20% of community-dwelling individuals ≥65 yrs and 18%–29% of patients ≥50 yrs in general medicine practices.[3] The American Heart Association estimates that 8–12 million Americans have PAD and that nearly 75% of those with PAD are asymptomatic.[3,4] To prevent severe complications and improve long-term prognosis, patients would benefit from the earliest possible identification of changes that suggest PAD. Early detection can lead to early treatment such as lifestyle modifications; exercise; smoking cessation; and antiplatelet, lipid-lowering, and antihypertensive pharmacotherapy.[5]

Diagnosis of PAD can be difficult because patients may not have clear symptoms initially. The Rose/World Health Organization, Edinburgh, and San Diego Claudication Questionnaires[5] have been used to diagnose PAD after claudication symptoms appear, but they are insensitive for detecting asymptomatic PAD.[6] The ankle-brachial index (ABI, ratio of systolic blood pressure at the ankle to that in the arm) has the greatest diagnostic accuracy of noninvasive diagnostic methods.[7] However, some limitations include the potential for recording falsely elevated ABIs in patients with diabetes with calcified arteries, the requirement of a skilled technician to operate the Doppler ultrasound equipment, and the inability to use (or perform the test) during exercise.[8]

Another noninvasive tool to detect PAD is near-infrared spectroscopy (NIRS). This method measures oxygenated and deoxygenated hemo/myoglobin and monitors the balance of oxygen (O_2) supply to O_2 demand in exercised muscles. Oxyhemo/myoglobin saturation of calf tissue with O_2 (StO_2; in percentage saturation) by NIRS, especially desaturation during exercise, has been used to diagnose PAD more accurately than ABI.[9–12]

Current clinical guidelines for vascular rehabilitation recommend walking as the exercise testing mode of choice.[1] However, many patients with PAD have conditions that preclude treadmill or surface walking exercise for testing and training, including unstable/severe coronary artery disease, congestive heart failure, hemiparesis, severe arthritis, chronic pain, leg amputation, balance impairment, and other disabling conditions. Gardner et al.[13] has reported that, of 905 veteran PAD patients evaluated for exercise research, 190 (20%) were excluded because of exercise intolerance from factors other than leg pain (e.g., severe coronary artery disease, dyspnea, poorly controlled hypertension, cancer, or renal/liver disease). Other common disabilities that preclude safe treadmill walking include back or leg pain (e.g., degenerative joint disease, spinal stenosis, radicular pain, gout), transfemoral amputation, open foot wounds, hemiplegia, and severe balance impairment. Therefore, substantial numbers of ambulatory PAD patients will not be candidates for treadmill testing and may benefit from calf exercise testing. Isolated calf exercise is an alternative exercise mode for these individuals.[14]

The purpose of this study was to compare calf tissue oxygenation responses to calf exercise in men with and without selected risk factors for developing PAD. We hypothesized that subjects with PAD risk factors would show greater oxyhemo/myoglobin desaturation during calf exercise compared with subjects without risk factors.

METHODS

Study Design

This study involved a cross-sectional quasi-experimental design involving comparisons between two groups and among changes in StO_2 (calf tissue % HbO_2 saturation) from resting baselines to three increasing exercise loads. The blinding of testers to the group membership of subjects was not possible.

Subjects

A convenience sample of 65 ambulatory men, ages 40–83 yrs, without diagnosed PAD, were recruited at a large urban tertiary medical center for veterans and from the surrounding community. Before screening and participation, the subjects signed an informed consent form that was approved by the medical center's institutional review board in accordance with the Declaration of the World Medical Association.

Of the 65 subjects, 20 were recruited from hospital staff, and the local community had no apparent risk factors for PAD (including age ≥65 yrs, type 2 diabetes mellitus, hypertension, hyperlipidemia, obesity, current tobacco use, or smoking

history ≥10 yrs). These subjects were originally recruited and tested for a previously published study of healthy subjects.[15] At that time, they had denied, on a self-report health survey, having any of the following conditions:

- acute medical illness such as a respiratory, local, or systemic infection;
- diabetes mellitus;
- hypertension;
- hyperlipidemia;
- lower-limb PAD, edema, or circulatory or neurologic abnormality;
- obesity (body mass index ≥30 kg/m^2);
- congestive heart failure;
- cerebrovascular accident;
- myocardial infarction;
- peripheral neuropathy;
- admitted cigarette smoking or illicit drug use within the past year;
- inability to perform resistive ankle plantar flexion exercise with at least one leg for 5 mins; and
- age ≥65 yrs.

The other 45 "at-risk" subjects were veterans recruited from the General Physical Medicine and Rehabilitation Clinic at the medical center. Prospective at-risk subjects were screened by self-report of at least one PAD risk factor with no acute illness or symptoms/history of claudication, PAD diagnosis, ABI of 0.90 or less, myocardial infarction, congestive heart failure, or stroke. After informed consent was completed, self-reported data were confirmed by review of subjects' electronic medical records. The risk factor of diabetes was diagnosed based on its documentation in the medical record, elevated hemoglobin A1c greater than 6.0, or currently prescribed diabetic medication. The risk factor of hypertension was diagnosed based on its documentation in the medical record, two separate occasions with blood pressure assessed as 140/90 mm Hg or higher, or currently prescribed antihypertensive medication. The risk factor of hyperlipidemia was diagnosed based on its documentation in the medical record, an elevated total serum cholesterol concentration greater than 200 mg/dl, serum low-density lipoprotein concentration greater than 100 mg/dL, serum triglyceride concentration greater than 160 mg/dl, or currently prescribed lipid-lowering medication. The risk factor of smoking was determined by patient self-report as currently smoking or at least a 10 pack-yr history. The risk factor of obesity was determined as having a body mass index of 30 kg/m^2 or higher. The risk factor of advanced age was defined as 65.0 yrs or older. These six risk factors were chosen based on their being independent risk factors for PAD and ease of assessment with available resources.

No at-risk subject had a diagnosis of PAD before screening and risk factor analysis, and none had an ABI of 0.90 or less. One at-risk subject had an ABI of 1.66, suggesting calcification of distal arteries but not diagnostic of PAD. This elevated ABI was considered invalid and not included in the group's descriptive statistics for ABI. Mean ± SD ABI of the at-risk group was 1.07 ± 0.08.

Testing Protocol

All testing was conducted in a quiet thermoneutral hospital laboratory. Before testing, subjects rested quietly in the laboratory for approximately 30 mins. The room temperature and relative humidity were measured before testing using a Thermo-Humidity Meter (Model 61161-3783; VWR LabShop, Batavia, IL). Each subject initially completed the International Physical Activity Questionnaire (short last 7 days self-administered format)[16] and Walking Impairment Questionnaire.[17] One leg was randomly chosen for study. Although the subject was standing, the leg to be tested was placed on a footstool while a researcher marked a point on the midline of the medial side of the leg at the level of the maximum girth of the calf. Skinfold thickness at the test site was measured by gently pinching a vertical skinfold and taking the average of three skinfold measurements using a Lange-type skinfold caliper (Therapeutic Instruments Inc., Clifton, NJ). Subjects were excluded if the skinfold was greater than 25.0 mm to ensure that substantial calf muscle tissue was sampled by the NIRS. The skin temperature was determined at the site of testing using a First Temp Genius Thermometer Model 3000A (Sherwood Medical, Crawley, Sussex, UK) because skin temperature less than 28°C would strongly suggest circulatory and/or neurologic abnormality. At-risk subjects' body mass was assessed before testing on a Scale-Tronix scale, Model 6002 Wheelchair Scale (Scale-Tronix Inc., White Plains, NY). The subjects self-reported their height without shoes to the nearest half-inch. Height, body mass, body mass index, and ABI were not obtained for "no-risk" subjects because those measurements were not part of the testing protocol in the previous study in which they participated.[14] To assess hypertension, each subject's left brachial blood pressure was measured using an electronic sphygmomanometer (Dinamap ProCare Auscultatory 400; The General Electric Company, GE Healthcare Division, Fairfield, CT) with appropriately

sized arm cuff and recorded before and after the testing protocol.

In addition, subjects with risk factors also underwent ABI testing and were administered the Edinburgh Claudication Questionnaire.[18] More exclusion criteria included positive Edinburgh Claudication Questionnaire, ABI of 0.9 or less, smoking within 2 hrs of testing, claudication during testing, medial calf skinfold greater than 25 mm, or inability to perform 8-W calf exercise with at least one leg for 5 mins.

The subjects assumed the semirecumbent position on a hospital bed with their shoes removed for approximately 30 mins before data collection. During this time, informed consent and questionnaires were completed, the protocol was explained, questions were answered, the subject was prepared for testing, and instrumentation was calibrated. A patch of skin (7 × 5 cm) was shaved on the medial calf at the test site, and a plastic adhesive shield was placed to block out ambient light and hold the 25-mm optical NIRS probe securely against the skin. The NIRS system was calibrated before each test as per manufacturer's instructions. The probe remained in place for the duration of the test (approximately 1 hr). The subjects performed a testing protocol consisting of seven consecutive 5-min test stages:

Stage 1: Baseline rest;
Stage 2: 0-W (non-resistive) active plantar/dorsiflexion exercise;
Stage 3: Rest/recovery;
Stage 4: 4-W resistive plantar flexion exercise;
Stage 5: Rest/recovery;
Stage 6: 8-W resistive plantar flexion exercise; and
Stage 7: Rest/recovery.

Stage 2 exercise was accomplished with the subject's heel positioned off the foot of the bed to minimize frictional resistance. For resistive 4- and 8-W exercise, a Stresst'er (Stu-ert Medical Devices Ltd., Frinton-on-Sea, Essex, UK) spring-loaded pedal ergometer[19] was mounted on an adjustable-height cart and rolled to the foot of the bed so the subject's foot could be placed on the pedal. The ergometer provided alternating concentric and eccentric gastroc-soleus contractions at 4 W (60 contractions per minute) and 8 W (120 contractions per minute), paced by a metronome. These exercise intensities were chosen to represent those which induce severe claudication in PAD patients.

StO_2 was digitally acquired at the medial calf site in 3- to 4-sec intervals using a near infrared spectrometer, the InSpectra Tissue Spectrometer, Model 325 (Hutchinson Technology Inc., Hutchinson, MN). This is a noninvasive monitoring system that measures an approximated percentage of oxyhemo/myoglobin saturation within a localized area of tissue, including skin, subcutaneous tissue, and muscle) to a depth of approximately 2 cm. NIRS measures oxyhemo/myoglobin oxygenation values in tissue based on spectrophotometric principles that relate light absorption to chemical concentration. Subjects were asked to report any leg muscle symptoms (such as pain or fatigue) and the intensity of those symptoms on the Borg CR-10 scale[14] at every minute during the rest and exercise phases.

We previously reported reliability coefficients for StO_2.[15] Eleven subjects without risk factors were randomly selected for retesting to assess the reliability of StO_2 and ΔStO_2 measurements at all test stages combined. Retests were conducted on different days within 2 wks of each other. Fasting (hemodynamic) state was not controlled, perhaps adding to the variability of test-retest data. The StO_2 and ΔStO_2 data from eleven subjects were normally distributed. For both StO_2 and ΔStO_2, the tests showed no statistically significant difference between mean StO_2 for trials 1 and 2. Intraclass correlation coefficients (model 2,k) were 0.88 and 0.77, respectively, for StO_2 and ΔStO_2. Bland-Altman plots revealed that all StO_2 data fell within ±2 standard deviations of the mean difference between trials 1 and 2. These tests and plots show that StO_2 and ΔStO_2 have moderate-to-high test-retest reliability.

Data Analysis

To minimize multiple testing error, the number of statistical hypothesis tests was minimized, and the Bonferroni correction was applied to the 14 statistical tests.[20] This correction serves to maintain the experiment-wise type I error rate at 0.05 ($\alpha = 0.05/14 = 0.0036$). Seven two-tailed independent t tests were used to determine differences in baseline characteristics between groups. StO_2 variables were calculated at the seven test stages as follows: the mean StO_2 during the last minute of rest before each exercise bout and the minimum StO_2 values during 0-, 4-, and 8-W exercise bouts. Change scores were calculated to represent the change (decrement) in StO_2 (ΔStO_2) during each exercise stage from the preceding resting baseline. Two repeated-measures analyses of variance across the four rest stages were computed to determine the differences among resting baseline StO_2 values for each group, to assess the adequacy of the 5-min recovery stages, and the degree of any postexercise

TABLE 1 Demographic data

	At-Risk Group (n = 45)		No-Risk Group (n = 20)	
	n	%	n	%
Ethnicity				
African American	19	42	5	21
White, non-Hispanic	16	36	13	62
Latino	6	13	1	4
Asian	2	4	1	12
Multiethnic	2	4	0	0
Education				
<High school diploma/GED	2	4	0	0
High school diploma/GED	6	13	0	0
Some college	27	60	4	25
At least one college degree	10	22	16	80

GED, General Educational Development certificate.

hyperemia. Three one-tailed Mann-Whitney U tests were performed to determine the differences between groups for ΔStO_2 during each exercise bout. Two repeated-measures analyses of variance and two intraclass correlation coefficients (model 2,k) were computed to assess the test-retest reliability of StO_2 and ΔStO_2 measurements. A Bland-Altman plot of differences between trials 1 and 2 as a function of the average of both trials[21] was also used to illustrate the variability of ΔStO_2 data in 11 randomly chosen healthy subjects.[20] NCSS 2005 (Kaysville, UT) statistical software was used to analyze data.

RESULTS

All subjects completed one or two trials of the seven-stage testing protocol without pain, claudication, difficulty, or adverse events of any kind, although some reported mild local (gastrocnemius-soleus) muscle fatigue that was completely resolved during rest.

Group Comparisons

Demographic and descriptive characteristics of subjects are shown in Tables 1 and 2. The no-risk group consisted of 20 men with a mean ± SD age of 55.4 ± 6.5 yrs. The at-risk group consisted of 45 men with a mean ± SD age of 62.8 ± 8.9 yrs. The two

TABLE 2 Descriptive statistics for the no-risk and at-risk groups

	No-Risk Group (n = 20)				At-Risk Group (n = 45)					
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum	Δ	P^a
Age, yrs	55.4	6.5	40.0	63.0	62.1	8.7	45.0	80.0	6.7	0.003
Physical activity, MET-mins/wk	3022	2231	0	7704	3512	3078	0	10,500	1823	0.523
Walking impairment: distance[b]	0.93	0.23	0.04	1.00	0.89	0.24	0.14	1.00	−3.9	0.540
Walking impairment: speed[b]	94	20	11	100	70	28	13	100	−28	<0.001
Walking impairment: stairs[b]	78	22	21	100	78	26	12	100	0	[c]
Ambient temperature, °C	23.1	10.2	21	54	23.1	0.3	23	24	−0.01	[c]
Ambient relative humidity, %	37.4	10.2	21	54	37.2	12.4	18	59	−0.3	[c]
Calf skin temperature, °C	30.8	0.8	29.0	32.1	29.2	0.9	26.9	31.4	−1.7	<0.001
Calf skinfold, mm	8.8	3.3	3.7	15.0	10.8	5.2	3.0	25.0	2.0	0.120
Systolic blood pressure, mm Hg	127	12	95	140	132	18	122	171	4.3	0.328
Diastolic blood pressure, mm Hg	79	9	63	95	78	8	62	97	−0.4	[c]
Height, cm	no data				178.2	71	160.0	190.0		
Body mass, kg					91.7	16.8	70.0	147.0		
Body mass index, kg/m^2					28.9	4.7	22.0	44.0		
Ankle-brachial index					1.09	0.12	0.90	1.66		

[a]P value for independent t tests.

[b]Percentage of maximal score.

[c]Independent t tests were not performed because of lack of clinical significance of the small differences between groups.

MET, metabolic equivalents or multiples of resting metabolic rate.

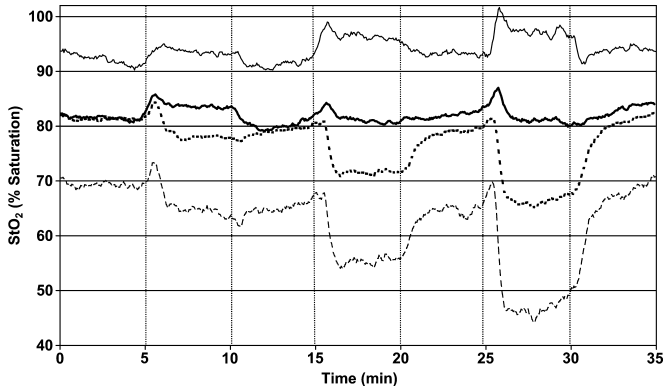

FIGURE 1 *Mean calf tissue oxygenation (StO_2) raw data for the no-risk (bold solid line) and at-risk (bold dashed line) groups at 3- to 4-sec intervals throughout the seven-stage testing protocol. Corresponding solid and dashed standard deviation lines are not in bold.*

groups were ethnically diverse; however, the at-risk group had a higher proportion of blacks (42%) compared with the no-risk subjects (21%). The no-risk group had a higher proportion of non-Hispanic whites (62%) compared with the at-risk group (34%). The at-risk group also had a lower level of educational attainment, with 10% having received at least one college degree *vs.* 80% of the no-risk group.

In Table 2, the at- and no-risk groups were virtually identical and not clinically significantly different on several pretest characteristics including stair-related walking impairment, diastolic blood pressure, and ambient temperature and relative humidity. Therefore, no statistical tests were performed to determine statistical differences between the groups on these variables. On the other variables, independent *t* tests were performed and showed that the at- and no-risk groups were not significantly different on physical activity, distance-related walking impairment, systolic blood pressure, and calf skinfold thickness.

Also in Table 2, independent *t* tests revealed that the at-risk group was significantly older (by 6.7 yrs, $P = 0.001$) than the no-risk group. The at-risk group reported significantly more difficulty walking at higher speeds ($P < 0.001$) compared with the no-risk group. Calf skin temperature before testing was significantly lower ($P < 0.001$) in the at-risk group (29.2°C) compared with the no-risk group (30.8°C).

The distribution of risk factors among at-risk subjects was as follows: 82% had hyperlipidemia; 67%, hypertension; 53%, current smokers or 10 pack-yr or longer history; 33%, 65 yrs or older; 29%, obesity; and 27%, type 2 diabetes mellitus. Five subjects in the at-risk group had one PAD risk factor; 13 had two; 12 had three; 9 had four; 5 had five; and one had 6.

Figure 1 illustrates the mean ± SD raw StO_2 responses for the no-risk and at-risk groups at 3- to 4-sec intervals throughout the seven-stage testing protocol. Compared with the no-risk group, the trend of progressive desaturation during exercise in

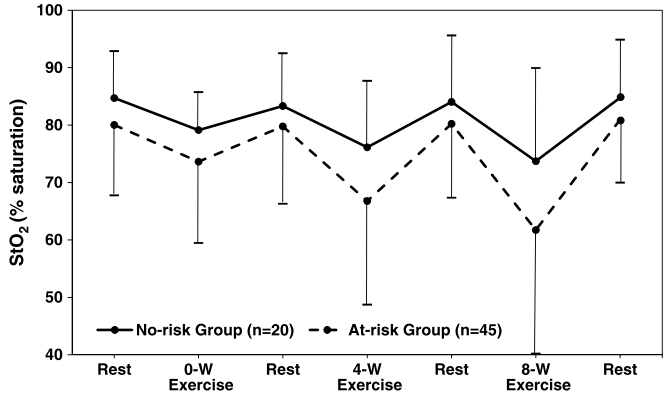

FIGURE 2 *Mean ± standard deviation calculated calf tissue oxygenation (StO_2) variables for the no-risk and at-risk groups at each test stage. Resting values represent the mean of StO_2 data during the fifth minute of rest; exercise values represent the mean StO_2-nadir values during exercise.*

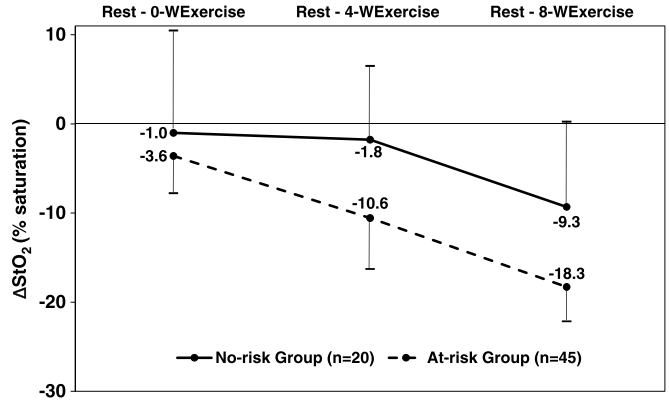

FIGURE 3 *Median ± interquartile range change in calculated calf tissue oxygenation (ΔStO_2) variables for each exercise stage (i.e., rest minus subsequent exercise stage) for the no-risk and at-risk groups.*

the at-risk group is striking. Substantial variability among subjects within each group is also evident.

Figure 2 shows the mean ± SD calculated StO_2 variables for the no-risk and at-risk groups at each test stage. Similar changes from rest to exercise and high within-group variability are apparent, especially for the at-risk group. The repeated measures analyses of variance revealed no significant differences among the four rest stages for either group (no-risk, $P = 0.747$; at-risk, $P = 0.514$).

The data for ΔStO_2 for the at-risk group were not normally distributed (strongly skewed to the left) and transformations of the data did not correct this; therefore, medians and interquartile ranges were used to represent measures of central tendency and variability for ΔStO_2. Figure 3 shows these median ± interquartile range ΔStO_2 values for each group for each of the three exercise bouts. The Mann-Whitney *U* tests revealed (1) a strong but nonsignificant trend ($P = 0.058$) toward the at-risk group having greater decrease in StO_2 during 0-W exercise and (2) ΔStO_2 (decrement) for the at-risk group being significantly greater than that for the no-risk group during 4- and 8-W exercise ($P < 0.001$ and $P = 0.002$, respectively).

DISCUSSION

Comparison of Characteristics of At- and No-Risk Groups

The most compelling and novel findings of this study were the greater decrements in calf oxygenation (ΔStO_2) induced by light-to-moderate (4- and 8-W) resistive calf exercise in subjects without diagnosed PAD but with risk factors for developing PAD. At-risk subjects' medial calf muscles desaturated about 9% more than the no-risk subjects. Similar findings have been demonstrated during treadmill exercise but not during calf exercise.[10]

Although much of these differences can be attributed to the PAD risk factors, some may possibly be caused by differences between the two groups. For example, the at-risk group was 6.5 yrs older than the no-risk group (age ≥65 yrs being a risk factor and exclusion criterion for no-risk subjects). It might be expected that decreased lifestyle physical activity and subsequent deconditioning would explain additional desaturation in the at-risk group, but no statistically significant differences in physical activity existed. In fact, the mean physical activity score for at-risk group was 60% higher than that for the no-risk group, but the high variability of scores in both groups ruled out any statistically significant difference. It may be that the International Physical Activity Questionnaire is not sensitive enough to detect differences between these groups.

Readers may wonder whether ethnic and educational differences between groups may have influenced the results because the at-risk group had a higher proportion of blacks as well as a higher proportion of those with lower educational attainment. Collins et al.[22] found a higher prevalence of PAD among black men compared with white or Latino men in a large sample including many American veterans. However, after adjusting for atherosclerotic risk factors (age, current smoking, diabetes, hypertension, and education), ethnicity was not an independent risk factor for PAD. Therefore, it is unlikely that ethnicity and education affect the results of this study.

Other differences between groups included lower speed-related walking impairment and calf skin temperature in the at-risk group, but these would seem to be effects of subclinical PAD instead of causes. The lack of clinically significant differences between groups of the ambient temperature and relative humidity in the testing laboratory rules

out the possibility that a colder room for the at-risk group affected skin temperature or perfusion. Unfortunately, lack of height, body mass, body mass index, and ABI measurements for the no-risk group leaves open the possibility that (1) higher body mass and body mass index were higher in the at-risk group or (2) some no-risk subjects had ABI values less than 0.90. However, these potential differences would not explain the greater calf muscle desaturation during exercise in the at-risk group. In general, the differences observed between groups do little to undermine the finding of greater decrements in calf oxygenation induced by calf exercise in subjects at-risk for developing PAD.

Comparison of Calf Oxygenation Responses of At- and No-Risk Groups

The finding of no significant differences among the four rest stages for either group indicates that the duration of the 5-min recovery stages were adequate for full recovery of the exercised calf muscles from the point of view of oxyhemo/myoglobin resaturation. Resaturation beyond baseline would have represented postexercise hyperemia and an undesirable non–steady-state condition before the subsequent exercise stage (i.e., carryover effect). This was a necessary prerequisite for a valid exercise test involving repeated exercise bouts and measurement of changes from stable pre-exercise baselines.

A major assumption of this study is that PAD risk factors are responsible for inducing excessive desaturation during exercise, suggesting the presence of PAD. Strong evidence supports the links between specific risk factors and excessive desaturation during exercise.[10,23–26] Atherosclerotic risk factors including hypertension, diabetes mellitus, smoking, and hyperlipidemia likely predispose the at-risk group to a variety of mechanisms that reduce capillary perfusion.[26,27] Smoking and diabetes damage vascular endothelial cells and blunt the release of factors that initiate the capillary vasodilatory response. Without vasodilation of the capillary beds, blood flow responses to increased tissue O_2 demand remain either static or blunted.[28–32] In addition to the vasodilatory response, the formation of atherosclerotic plaques present mechanical obstruction to blood flow and indicate preclinical PAD.

The results from our study reveal significantly greater StO_2 decrement at the two higher power outputs (4 and 8 W) compared with the 0-W exercise. This suggests that 0-W exercise is insufficiently intense to induce excessive desaturation that may indicate preclinical PAD. Therefore, workloads of at least 4 W should be used to detect unusual or excessive desaturation. It is not clear whether workloads greater than 8 W might be more appropriate to this end. Cheetham et al.[31] has suggested that 8.5-W load with the Stresst'er™ ergometer (as in the current study) is the optimal load to induce a fall in postexercise ankle systolic pressure higher than 30 mm Hg to diagnose PAD in a manner similar to more traditional treadmill exercise tests. Compared with treadmill walking, calf ergometry has the advantage of being independent of an obese subject's body mass, thus targeting the calf muscles with specific exercise loads and O_2 demands.

PAD Risk Factors

Several independent risk factors for PAD have been identified in PAD patients, including cigarette smoking, diabetes mellitus, hypertension, hyperlipidemia, obesity, advanced age, black ethnicity, and biochemical markers related to inflammation.[32,33] Some of these risk factors have been linked to excessive oxyhemo/myoglobin desaturation in ischemic muscles of PAD patients during exercise. Wallace et al.[12] first documented a relationship between risk factors and severity of PAD and increased calf muscle desaturation by NIRS during treadmill walking. Four groups of subjects included (1) healthy subjects, (2) subjects with risk factors for developing PAD (i.e., with hypertension, diabetes, hyperlipidemia, premature atherosclerosis, and/or current/former smoking history), (3) subjects with diagnosed PAD and intermittent claudication, (4) and subjects with PAD-related rest pain. Each subject performed a constant-load treadmill walking protocol (2 mph, 3% incline for 5 mins or until pain ensued), and changes in calf oxygenation were recorded. Mean calf oxygenation decreased by progressively greater amounts in groups 1 to 4. They suggested a 10% desaturation as a threshold between normal and abnormal response.

More recently, Afaq et al.[23] reported that compared with PAD patients who had not smoked for at least 1 year, although similar at rest, patients with PAD who currently smoked cigarettes had lower calf tissue oxygenation values after 1 and 2 mins of treadmill walking and at the onset of claudication. Similarly, compared with PAD patients without hypercholesterolemia, those with hypercholesterolemia had lower calf tissue oxygenation values during treadmill exercise at the onset of claudication and during maximum-tolerated claudication.[25] Gardner et al.[25] determined that PAD patients with more metabolic syndrome components (abdominal obesity, elevated fasting glucose, elevated triglycerides, reduced high-density lipoprotein cholesterol,

hypertension) have worsened intermittent claudication, physical function, health-related quality-of-life, and peripheral circulation. Abdominal obesity and elevated fasting glucose were the factors that were most predictive of these outcome measures.

It is known that smoking causes not only impaired endothelial function leading to atherosclerosis[34] but also partial transformation of hemoglobin to inactive carboxyhemoglobin.[35] Therefore, the decreased saturation observed in this study may result not only from PAD risk factors but also the inactivation of hemoglobin and resulting decreased ability for oxygenation. However, some evidence suggests that the effect of carboxyhemoglobin may be exaggerated. Kambam et al.[35] determined that high carboxyhemoglobin levels (6.6%) decreased to normal values (1.1%) within 12 hrs after smoking cessation (normal carboxyhemoglobin values were 0%–2% in nonsmokers). Of the 28 at-risk subjects with the smoking risk factor, 15 reported that they had smoked a cigarette within the past 11 hrs. Mean ± SD number of cigarettes smoked/day was 9.9 ± 6.3 (2–20 cigs/day), and the time since last cigarette was 3.2 ± 2.7 hrs (2–11 hrs). StO_2 baseline averaged 82.4% ± 12.3% saturation for active smokers (n = 15) and 82.6% ± 10.7% saturation for nonsmokers (n = 40), with no clinically significant difference between active smokers and nonsmokers. Therefore, greater StO_2 decrement in the at-risk group is not likely to be caused by high carboxyhemoglobin levels in the smoking subjects.

Calf Exercise

Treadmill testing is the most common exercise mode for evaluating claudication in ambulatory PAD patients. However, substantial numbers of PAD patients will not be candidates for treadmill testing because of the accumulation of comorbidities in time.[13] Calf exercise from the preclinical through severe disability PAD stages would have the advantage of maintaining a consistent exercise testing protocol throughout the natural course of PAD should it progress to severe morbidity. In this way, serial tests could be directly comparable with each other. Therefore, isolated calf ergometry as used in this study provides such an alternative exercise mode that would fulfill this need.

Limitations

There are several potentially confounding factors in this study. PAD risk factor determination for the no-risk group lacked rigor because it depended on the self-report of known conditions and risk factors (i.e., knowledge and honesty). Therefore, the absence of the risk factors and an abnormal ABI in the no-risk group could not be absolutely verified. If risk factors were underreported in the no-risk group, their StO_2 decrements during exercise may be slightly more abnormal than they should have been. It would be useful to better quantify physical activity/fitness of subjects to control for possibly confounding influence of this variable on StO_2 desaturation during exercise.

The findings of at-risk subjects in this study are applicable to men with one to six specific PAD risk factors. The results may have differed if other risk factors or women were included. It is acknowledged that other risk factors have been identified in the literature for men and women but that the relative importance and independence of each is the subject of ongoing research. In addition, there is no attempt to qualify results based on the number of risk factors per subject or relative contribution of each.

Future Research

Future research should investigate (1) the subset analysis of each risk factor and combinations thereof, (2) the identification of diagnostic standards for inadequate tissue oxygenation/saturation for specific O_2 demands, (3) the optimization of the exercise protocol using a higher workload, (4) the determination specificity and sensitivity of this test, and (5) comparison of NIRS-based variables with traditional clinical evaluations and other technologies. In addition, longitudinal research should determine the actual risk of various factors for the development of PAD symptoms and co-morbidities.

Only longitudinal research can determine whether StO_2 responses to exercise can detect diagnosable PAD. Additional research is necessary to determine normal and abnormal cutoff responses to specific workloads and O_2 demands values that are sensitive and specific to diagnosing PAD or to discriminate among severities of PAD (i.e., Fontaine claudication stages).[10]

CONCLUSIONS

Men with risk factors for developing PAD but without diagnosed PAD demonstrated greater decrements in calf oxygenation (ΔStO_2) during light-to-moderate calf exercise compared with men without those risk factors. Further development of this test may lead to early PAD diagnosis and intervention to modify risk factors and prevent PAD

comorbidities such as claudication, gangrene, amputation, coronary artery disease, renal insufficiency, and stroke.

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