Published in final edited form as:

Angiology. 2015 October ; 66(9): 867–874. doi:10.1177/0003319714566863.

ENDOTHELIAL CELL INFLAMMATION AND ANTIOXIDANT CAPACITY ARE ASSOCIATED WITH EXERCISE PERFORMANCE AND MICROCIRCULATION IN PATIENTS WITH SYMPTOMATIC PERIPHERAL ARTERY DISEASE

Andrew W. Gardner^{a,d}, Donald E. Parker^b, Polly S. Montgomery^a, Danuta Sosnowska^a, Ana I. Casanegra^c, Zoltan Ungvari^a, Anna Csiszar^a, and William E. Sonntag^a

^aReynolds Oklahoma Center on Aging, Donald W. Reynolds Department of Geriatric Medicine, University of Oklahoma Health Sciences Center (OUHSC), Oklahoma City, OK

^bDepartment of Biostatistics and Epidemiology, OUHSC, Oklahoma City, OK

^cCardiovascular Section, Department of Medicine, OUHSC, Oklahoma City, OK

^dVeterans Affairs Medical Center, Oklahoma City, OK

Abstract

We determined whether exercise performance and lower extremity microcirculation were associated with endothelial cell inflammation, oxidative stress, and apoptosis, and with circulating biomarkers of inflammation and antioxidant capacity in 160 patients with symptomatic peripheral artery disease (PAD). In a multivariate regression model for peak walking time PWT, significant independent variables included ankle-brachial index ($p < 0.001$), age ($p = 0.017$), hydroxyl radical antioxidant capacity ($p = 0.008$), and endothelial cell NF- κ B activity ($p = 0.015$). In multivariate analyses for time to minimum exercise calf muscle hemoglobin oxygen saturation (StO_2), significant independent variables included endothelial cell NF- κ B activity ($p = 0.043$), and calf muscle StO_2 at rest ($p = 0.007$). Endothelial cell inflammation and circulating biomarkers of inflammation and antioxidant capacity were associated with exercise performance and microcirculation of the ischemic calf musculature during exercise. The clinical implication is that interventions designed to alleviate endothelial cell inflammation and circulating inflammatory biomarkers, such as antioxidant therapy, may improve exercise performance of symptomatic patients with PAD.

Keywords

Antioxidant Capacity; Claudication; Inflammation; Peripheral Artery Disease

Address correspondence to: Andrew W. Gardner, Ph.D., Professor and Donald W. Reynolds Chair in Aging Research, OUHSC, Donald W. Reynolds Department of Geriatric Medicine, 1122 N.E. 13th Street, O'Donoghue Research Building, Suite 1200, Oklahoma City, OK 73117. Telephone (405) 271-8558 ext. 42743, FAX: 405-271-2882, andrew-gardner@ouhsc.edu.

Declaration of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

INTRODUCTION

Peripheral artery disease (PAD) is a highly prevalent,^{1–3} costly,^{4–7} and deadly condition.^{8, 9} Furthermore, PAD is debilitating, as patients have ambulatory dysfunction,¹⁰ impaired functional performance,^{11, 12} low physical activity levels,^{13, 14} and increased rates of functional decline and mobility loss compared to those without PAD.^{11, 12, 15} In addition, we have recently shown that patients with PAD have higher levels of inflammation and lower levels of vascular endothelial growth factor-A than healthy controls,¹⁶ and in controls with a high burden of co-morbid conditions and cardiovascular risk factors.¹⁷ The higher levels of circulating inflammatory markers in patients with PAD are associated with low intensity of community-based daily ambulation (i.e., cadence),¹⁸ low amounts of daily ambulation,¹⁸ poor functional performance at baseline^{19, 20} and functional decline over time.²¹ However, it is not clear whether a heightened pro-inflammatory state in the circulation of symptomatic patients with PAD is associated with impairments in key outcome measures of exercise performance during a standardized treadmill test, such as claudication onset time (COT), peak walking time (PWT), and lower extremity microcirculation, as measured by calf muscle hemoglobin oxygen saturation (StO₂). One potential defense to guard against negative consequences of inflammation is having higher levels of antioxidant capacity, but the influence of antioxidant capacity on exercise performance measures in symptomatic patients with PAD has not been examined.

In addition to the roles that systemic inflammation and antioxidant capacity may have on exercise performance and the microcirculation, biomarkers from the vasculature may have a stronger association with symptoms in patients with PAD. Endothelial dysfunction is an early marker of structural vascular changes and clinical symptoms,^{22–28} contributes to the progression of atherosclerosis,²⁸ and increases the risk of coronary events.^{22, 24, 28–30} We³¹ and others²⁸ have found that endothelial function is impaired in patients with PAD. However, the association that endothelial cell function has with exercise performance and the microcirculation of symptomatic patients with PAD is less clear.

The aim of the study was to determine whether exercise performance and lower extremity microcirculation were associated with endothelial cell inflammation, oxidative stress, and apoptosis, and with circulating biomarkers of inflammation and antioxidant capacity. We hypothesized that worse exercise performance and impaired lower extremity microcirculation are associated with greater endothelial inflammation, cellular reactive oxygen species (ROS) production, and apoptosis, and with higher levels of circulating inflammatory biomarkers and lower levels of circulating antioxidant capacity.

METHODS

Patients

Approval and Informed Consent—The institutional review board at the University of Oklahoma Health Sciences Center (HSC), and the Research and Development committee at the Oklahoma City VA Medical Center approved the procedures of this study. Written informed consent was obtained from each patient at the beginning of investigation.

Recruitment—Vascular labs and vascular clinics from the University of Oklahoma HSC and the Oklahoma City VA Medical Center referred patients for possible enrollment into an exercise rehabilitation program to treat leg pain secondary to PAD.³²

Medical Screening through History and Physical Examination

Patients were evaluated in the morning at the Clinical Research Center (CRC), at the University of Oklahoma HSC. Patients arrived fasted, but were permitted to take their usual medications. To begin the study visit, patients were evaluated with a medical history and physical examination in which demographic information, height, weight, waist circumference,³³ cardiovascular risk factors, co-morbid conditions, claudication history, ankle-brachial index (ABI), blood samples, and a list of current medications were obtained.

Inclusion and Exclusion Criteria—Patients with PAD were included in this study if they met the following criteria: (a) a history of ambulatory leg pain, (b) ambulatory leg pain confirmed by treadmill exercise,¹⁰ and (c) an ABI ≥ 0.90 at rest² or ≥ 0.73 after exercise.³⁴ Although there has been recent debate regarding the value of ABI as a measure to screen for PAD,^{35, 36} our decision to measure ABI both at rest and after treadmill exercise increases the sensitivity for detecting PAD, and differentiates between vascular claudication and pseudoclaudication, supported by a class 1 recommendation with level B evidence.² Another commonly used noninvasive vascular test to assess PAD is the determination of pulse volume recordings via photoplethysmography,^{37–39} but this technique is only supported by a class IIa recommendation with level B evidence.²

Patients were excluded for the following conditions: (a) absence of PAD (ABI > 0.90 at rest and ABI > 0.73 after exercise), (b) non-compressible vessels (ABI > 1.40), (c) asymptomatic PAD, (d) use of medications indicated for the treatment of claudication (cilostazol or pentoxifylline) initiated within 3 months prior to investigation, (e) exercise limited by other diseases or conditions, (f) active cancer, (g) end stage renal disease defined as stage 5 chronic kidney disease, and, (h) abnormal liver function. A consecutive series of 225 individuals were evaluated for eligibility, and 160 patients were deemed eligible for inclusion into the study and 65 patients were ineligible.

Measurements

Graded Treadmill Test: COT, PWT, and Calf Muscle StO₂—Patients performed a graded treadmill test to determine study eligibility, and to obtain outcome measures.¹⁰ The COT and PWT were measured, and are highly reliable, as previously described.¹⁰ Calf muscle StO₂ was measured with the treadmill test using a continuous-wave, NIRS unit (InSpectra model 325; Hutchinson Technology, Inc, Hutchinson, MN), an optical cable attached to a 25-mm probe, InSpectra software (version 2.0), and a dedicated laptop computer as previously described.⁴⁰

Blood Sampling—Blood was drawn by venipuncture from an antecubital vein, collected in vacutainers, and distributed in 0.5 ml aliquots. The samples were stored at -80°C , and were subsequently batched for analysis in duplicate in our laboratory at the Reynolds Oklahoma Center on Aging.

Endothelial Cell Cultures—A cell culture-based bioassay approach utilizing cultured primary human arterial endothelial cells was used to characterize the endothelial effects of circulating factors present in the sera of patients. In brief, endothelial cells (purchased from Cell Applications, Inc., San Diego, CA, after passage 4; age of the donors is unknown) were initially cultured in MesoEndo Endothelial Cell Growth Medium (Cell Applications, Inc) followed by Endothelial Basal Medium supplemented with 10% fetal calf serum until the time of serum treatment, as described.^{17, 41} Cells were 80–90% confluent before treatment with sera. Inter-individual variance is unlikely to contribute to observed differences because detector cells used for each in vitro study were from the same donor. For treatment, fetal calf serum was replaced with serum (10%; for 24–48 h) collected from our patients.¹⁷ Cells cultured in Endothelial Basal Medium supplemented with 10% fetal calf serum served as an additional control.

Apoptosis Assay—Cultured endothelial cells were treated with sera from patients for 24 hours. Caspase activities using Caspase-Glo 3/7 assay kit (Promega, Madison, WI) were measured to assess apoptotic cell death, as previously reported.¹⁷

Cellular ROS Production—Hydrogen peroxide (H₂O₂) production in detector endothelial cells was measured fluorometrically by a Tecan Infinite M200 (Tecan Group Ag, Mannedorf, Switzerland) using the Amplex Red/horseradish peroxidase assay (Life Technologies, Grand Island, NY) to determine cellular oxidative stress induced by factors in present in the sera.¹⁷

Transient Transfection, Nuclear Factor K-Light-Chain-Enhancer of Activated B Cells (NF-κB) Reporter Gene Assay—Transcriptional activity of NF-κB was tested in serum-treated detector endothelial cells by a reporter gene assay, to determine cellular pro-inflammatory effects induced by factors in the sera.¹⁷ Transfections in endothelial cells were performed using the Amaxa Nucleofector technology (Amaxa, Gaithersburg, MD), as we have previously reported.¹⁷

Serum Antioxidant Capacity—Hydroxyl Radical Antioxidant Capacity (HORAC) using the OxiSelect HORAC Activity Assay (Cell Biolabs Inc., San Diego, CA) was measured from sera to determine the capacity of antioxidant enzymes and other redox molecules to counterbalance the deleterious effects of oxidative stress in the sera of patients.¹⁷

Circulating inflammatory and vascular biomarkers—A Milliplex Human Adipokine Magnetic Bead Kit was used for determining tumor necrosis factor alpha (TNF α), interleukin-1b (IL-1b), interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), hepatocyte growth factor (HGF), and nerve growth factor (NGF). A Milliplex Human Cardiovascular Disease (CVD) Panel 1 Kit was used for myeloperoxidase (MPO), matrix metalloproteinase 9 (MMP-9), E selectin, vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), and plasminogen activity inhibitor-1 (PAI-1). A Milliplex Human Apolipoprotein Kit was used for apolipoprotein B and apolipoprotein CIII. The Milliplex kits were purchased from EMD Millipore, Billerica, MA. Affymetrix Procarta Immunoassay was used to detect serum amyloid A (SAA), vascular endothelial growth factor-A (VEGF-A) and adiponectin. These assays were

performed according to manufacturer's protocols by a Bio-Plex 200 System, Bio Rad, CA at the Core Facility, University of Oklahoma Health Sciences Center). Sample protein content was determined for normalization purposes by a spectrophotometric quantification method using BCA reagent (Pierce Chemical Co., Rockford, IL).

High Sensitivity C-Reactive Protein (HsCRP)—Concentration of HsCRP was quantified from 300 μ l of sera using a high-sensitivity Near Infrared Particles Immunoassay. The SYNCHRON LX-20 (Beckman-Coulter; California, USA), a commercially available device, was used to perform the assay. Prior to performing each assay, the SYNCHRON system was calibrated, and a calibration curve was established.⁴²

Statistical Analyses

Measurement variables were summarized in Tables I and II by reporting means and standard deviations and dichotomized variables by reporting percentages. Associations reported in Table III are Pearson partial correlation coefficients or Spearman partial correlation coefficients (indicated in italicized print), controlled for age, sex, race, ABI, current smoking, body mass index, and obesity. Spearman was reported if the two correlations differ by more than .1. A stepwise regression procedure with entry p value set at .05 was used to obtain regression models of Table IV.

RESULTS

The clinical characteristics of 160 patients with claudication are shown in Table 1. The group consisted of a mix of older, abdominally obese Caucasian and African-American men and women. Cardiovascular risk factors were highly prevalent in the group, particularly hypertension, dyslipidemia and metabolic syndrome. Consequently, 83% of patients were treated for hypertension, 70% were treated for dyslipidemia with statin medication, and 39% were treated for diabetes.

The calf muscle StO₂ measures of the group are shown in Table 2. On average, calf muscle StO₂ decreased 70% from rest to the mean minimum value attained 204 seconds after the initiation of exercise. At the completion of exercise, the mean time for calf muscle StO₂ to increase to half of the resting value was 155 seconds, whereas full recovery was reached at 231 seconds. Calf muscle StO₂ continued to increase to an average maximum value of 79% saturation 669 seconds after completion of exercise.

The correlation coefficients between measures of exercise performance and measures of circulating antioxidant capacity, inflammation, and cultured endothelial cells treated with sera in patients with PAD are shown in Table 3. After adjusting for age, sex, race, ABI, current smoking, BMI, and obesity, PWT was correlated with HsCRP ($p < 0.05$), HORAC ($p < 0.01$), VCAM-1 ($p < 0.05$), and endothelial cell NF- κ B activity ($p < 0.001$). Furthermore, COT and the time to minimum exercise calf StO₂ were correlated with endothelial cell NF- κ B activity, ($p < 0.01$ and $p < 0.05$, respectively), and the average rate of decline in calf StO₂ was correlated with hsCRP ($p < 0.01$), HORAC ($p < 0.05$), and endothelial cell NF- κ B activity ($p < 0.05$).

Multivariate regression models to predict exercise performance are shown in Table 4. For COT the significant independent variable was endothelial cell NF- κ B activity ($p = 0.013$), and for PWT the significant independent variables included ABI ($p < 0.001$), age ($p = 0.017$), HORAC ($p = 0.008$), and endothelial cell NF- κ B activity ($p = 0.015$). For the time to minimum exercise calf muscle StO₂, the significant independent variables included the calf muscle StO₂ measured at rest ($p = 0.007$), and endothelial cell NF- κ B activity ($p = 0.043$), and for the average rate of decline in calf muscle StO₂, the significant independent variables included BMI ($p < 0.001$) and HORAC ($p = 0.024$).

DISCUSSION

The primary novel finding was that higher endothelial cell NF- κ B activity was associated with lower PWT, lower COT, and shorter time to minimum calf muscle StO₂ during exercise. Another novel finding was that higher levels of circulating hsCRP and VCAM-1 were associated with lower PWT, and that higher circulating HORAC was associated with higher PWT and with slower average rate of decline in calf muscle StO₂ during exercise. The clinical significance is that new treatments designed to reduce endothelial cell inflammation and circulating inflammatory biomarkers, and to increase antioxidant capacity, might be efficacious to improve microvascular function and ambulation in patients with PAD. New treatments could include various antioxidant supplements to reduce inflammation and oxidative stress, to be taken in combination with, or separate from, exercise therapy. Increased intake of antioxidants could also occur from dietary intervention designed to consume fruits and vegetables rich in antioxidants. Statin medications could be another treatment approach, as they have been found to improve ambulation in patients with PAD,⁴³ and we have found that apolipoprotein sub-particle profiles are better in PAD patients who are prescribed statins.⁴⁴ However, it is not clear whether statin therapy would alter the inflammatory biomarkers measured in this study. The methodology of this study utilizing the Gardner-Skinner graded treadmill protocol to measure COT and PWT¹⁰ with concomitant measurement of calf muscle oxygenation using near-infrared spectroscopy, along with the assays described in this report, would be appropriate techniques to assess efficacy of new treatments designed to improve inflammation, ambulation, and microvascular function in patients with PAD.

Endothelial Cell NF- κ B activity was Negatively Associated with Exercise Performance and Calf Muscle StO₂

This is the first study to report that endothelial cell NF- κ B activity was associated with PWT and COT during standardized treadmill exercise in symptomatic patients with PAD, as well as with calf muscle StO₂ during exercise. Thus, endothelial cell inflammation is associated with worse leg symptoms and microcirculation of the ischemic calf musculature. The negative association between endothelial cell NF- κ B activity and PWT was significant even after adjusting for ABI, age, and HORAC levels. This finding suggests that endothelial cell NF- κ B activity may be associated with peak exercise performance independent of the severity of PAD in the large arteries, age, and antioxidant capacity. This observation was confirmed by noting that higher endothelial cell NF- κ B activity was also associated with shorter COT and with faster oxygen desaturation of the calf musculature during exercise.

Collectively, our results indicate that endothelial cell inflammation was negatively associated with exercise performance in symptomatic patients with PAD.

Circulating Inflammatory Biomarkers were Negatively Associated with Exercise Performance and Calf Muscle StO₂

Another observation was that after adjusting for age, sex, race, ABI, current smoking, BMI, and obesity, PWT was correlated with HsCRP and VCAM-1, and the average rate of decline in calf StO₂ also was associated with HsCRP. Our results indicate that circulating inflammatory biomarkers were negatively associated with both leg symptoms and the microcirculation of the ischemic calf musculature. These findings support an earlier study from our laboratory that daily ambulatory cadence as well as the total number of daily strides and minutes spent ambulating were associated with HsCRP in symptomatic patients with PAD.¹⁸ The current study also supports work that higher levels of circulating inflammatory biomarkers consisting of hsCRP, IL-6, VCAM-1, ICAM-1, homocysteine, and D-dimer were associated with poor baseline functional performance in patients with PAD, as measured by 6-minute walk distance, pace of walking four meters, and the short physical performance battery.^{19, 20} Furthermore, greater inflammation was associated with decline in the short physical performance battery in patients with PAD over time.²¹ Collectively, the results from our current study and previous studies suggest that circulating biomarkers of inflammation were negatively associated with daily ambulatory activity and physical function. However, it should be noted that in the current study, none of the circulating inflammatory biomarkers entered the multivariate models as predictors of PWT, COT, and calf muscle StO₂, and therefore they were less strongly associated with exercise performance than the inflammatory measure of endothelial cell NF-κB activity.

Circulating Antioxidant Capacity was Positively Associated with Exercise Performance and Calf Muscle StO₂

This is the first study to report that higher circulating HORAC values were correlated with PWT during standardized treadmill exercise, and with a slower average rate of decline in calf muscle StO₂ during exercise. Thus, circulating antioxidant capacity was positively related to both leg symptoms and the microcirculation of the ischemic calf musculature during exercise. For PWT, the positive association with HORAC was evident after adjusting for ABI, age, and endothelial cell NF-κB activity. Consequently, higher circulating HORAC levels were associated with better ambulation independent of the severity of PAD, age, and endothelial cell inflammation in symptomatic patients. Furthermore, higher circulating HORAC levels were correlated with slower oxygen desaturation of the calf musculature during exercise after adjusting for BMI, suggesting that antioxidant capacity was positively related to the microcirculation of the calf musculature in symptomatic patients regardless of whether they were obese, overweight, or of normal weight. Collectively, our results suggest that interventions designed to increase HORAC levels may improve claudication and the calf microcirculation. An important source of antioxidants is dietary intake of fruits and vegetables. We have previously reported that patients with PAD have diets low in fruits and vegetables, indicated by low intake of fiber, as only 26% of patients met the daily recommendation for dietary fiber.⁴⁵ There is a relationship between increased fiber intake

and lower hsCRP,⁴⁶ suggesting that greater fruit and vegetable consumption may enhance HORAC and thereby lower inflammation.

Limitations

There are limitations to this study. A self-selection bias may exist regarding study participation, as patients who participated in this trial were volunteers. Therefore, they may represent those who were more interested in participation, who had better access to transportation to the research center, and who had relatively better health than patients who did not volunteer. Furthermore, the results of this study are only applicable to symptomatic patients with PAD, and may not be generalized to asymptomatic patients and patients with more severe forms of PAD, such as critical limb ischemia. Another limitation is that we did not have a healthy control group to examine whether relationships exist among inflammation, antioxidant capacity, exercise performance, and microcirculation, or whether these relationships are unique to patients with PAD. Finally, there are limitations associated with the design of the study. Significant association found among variables in the patients measured at baseline does not provide evidence of causality. Although these limitations exist, we believe that the findings of the present study are generalizable to the large number of symptomatic patients with PAD because women and African-Americans are well represented, and typical risk factors for PAD such as dyslipidemia, hypertension, obesity, diabetes, and smoking are highly prevalent.

Conclusion and clinical significance

Endothelial cell inflammation and circulating biomarkers of inflammation and antioxidant capacity were associated with exercise performance and microcirculation of the ischemic calf musculature during exercise. The clinical implication is that interventions designed to alleviate endothelial cell inflammation and circulating inflammatory biomarkers, such as antioxidant therapy, may improve exercise performance of symptomatic patients with PAD.

Acknowledgments

Supported by grants from the National Institute on Aging (R01-AG-24296 to AWG; AG031085 to AC; AG038747 to WES), Oklahoma Center for the Advancement of Science and Technology (to AWG, AC, WES and ZU), and General Clinical Research Center (M01-RR-14467), the American Heart Association (to AC and ZU), the National Center for Complementary and Alternative Medicine (R01-AT006526 to ZU) and the Ellison Medical Foundation (to WES).

References

1. 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. [PubMed: 23915883]
2. Hirsch AT, Haskal ZJ, Hertzner NR, Bakal CW, Creager MA, Halperin JL, et al. Acc/Aha 2005 Practice Guidelines for the Management of Patients with Peripheral Arterial Disease (Lower Extremity, Renal, Mesenteric, and Abdominal Aortic): A Collaborative Report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the Acc/Aha Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients with Peripheral Arterial Disease): Endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart,

- Lung, and Blood Institute; Society for Vascular Nursing; Transatlantic Inter-Society Consensus; and Vascular Disease Foundation. *Circulation*. 2006; 113:e463–e654. [PubMed: 16549646]
3. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG. Inter-Society Consensus for the Management of Peripheral Arterial Disease (Tasc II). *J VascSurg*. 2007; 45(Suppl S):S5–67.
 4. Hirsch AT, Hartman L, Town RJ, Virnig BA. National Health Care Costs of Peripheral Arterial Disease in the Medicare Population. *Vasc Med*. 2008; 13:209–15. [PubMed: 18687757]
 5. Jaff MR, Cahill KE, Yu AP, Birnbaum HG, Engelhart LM. Clinical Outcomes and Medical Care Costs among Medicare Beneficiaries Receiving Therapy for Peripheral Arterial Disease. *Ann Vasc Surg*. 2010; 24:577–87. [PubMed: 20579582]
 6. Mahoney EM, Wang K, Keo HH, Duval S, Smolderen KG, Cohen DJ, et al. Vascular Hospitalization Rates and Costs in Patients with Peripheral Artery Disease in the United States. *Circ Cardiovasc Qual Outcomes*. 2010; 3:642–51. [PubMed: 20940249]
 7. Sachs T, Pomposelli F, Hamdan A, Wyers M, Schermerhorn M. Trends in the National Outcomes and Costs for Claudication and Limb Threatening Ischemia: Angioplasty Vs Bypass Graft. *J Vasc Surg*. 2011; 54:1021–31. e1. [PubMed: 21880457]
 8. Brass EP, Hiatt WR. Review of Mortality and Cardiovascular Event Rates in Patients Enrolled in Clinical Trials for Claudication Therapies. *VascMed*. 2006; 11:141–5.
 9. Criqui MH, Langer RD, Fronek A, Feigelson HS, Klauber MR, McCann TJ, et al. Mortality over a Period of 10 Years in Patients with Peripheral Arterial Disease. *N Engl J Med*. 1992; 326:381–6. [PubMed: 1729621]
 10. Gardner AW, Skinner JS, Cantwell BW, Smith LK. Progressive Vs Single-Stage Treadmill Tests for Evaluation of Claudication. *Med Sci Sports Exerc*. 1991; 23:402–8. [PubMed: 2056896]
 11. 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. [PubMed: 11585483]
 12. 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. [PubMed: 15280343]
 13. McDermott MM, Liu K, O'Brien E, Guralnik JM, Criqui MH, Martin GJ, et al. Measuring Physical Activity in Peripheral Arterial Disease: A Comparison of Two Physical Activity Questionnaires with an Accelerometer. *Angiology*. 2000; 51:91–100. [PubMed: 10701716]
 14. Sieminski DJ, Gardner AW. The Relationship between Free-Living Daily Physical Activity and the Severity of Peripheral Arterial Occlusive Disease. *VascMed*. 1997; 2:286–91.
 15. McDermott MM, Greenland P, Liu K, Guralnik JM, Celic L, Criqui MH, et al. The Ankle Brachial Index Is Associated with Leg Function and Physical Activity: The Walking and Leg Circulation Study. *Ann Intern Med*. 2002; 136:873–83. [PubMed: 12069561]
 16. Gardner AW, Parker DE, Montgomery PS, Sosnowska D, Casanegra AI, Esponda OL, et al. Impaired Vascular Endothelial Growth Factor a and Inflammation in Patients with Peripheral Artery Disease. *Angiology*. 2014; 65:683–90. [PubMed: 24006146]
 17. Gardner AW, Parker DE, Montgomery PS, Sosnowska D, Casanegra AI, Ungvari Z, et al. Greater Endothelial Apoptosis and Oxidative Stress in Patients with Peripheral Artery Disease. *Int J Vasc Med*. 2014; 2014:160534. [PubMed: 24963409]
 18. Gardner AW, Parker DE, Montgomery PS, Blevins SM, Teague AM, Casanegra AI. Monitored Daily Ambulatory Activity, Inflammation, and Oxidative Stress in Patients with Claudication. *Angiology*. 2013; 65:491–6. [PubMed: 23695338]
 19. McDermott MM, Greenland P, Green D, Guralnik JM, Criqui MH, Liu K, et al. D-Dimer, Inflammatory Markers, and Lower Extremity Functioning in Patients with and without Peripheral Arterial Disease. *Circulation*. 2003; 107:3191–8. [PubMed: 12810614]
 20. McDermott MM, Liu K, Ferrucci L, Tian L, Guralnik JM, Green D, et al. Circulating Blood Markers and Functional Impairment in Peripheral Arterial Disease. *J Am Geriatr Soc*. 2008; 56:1504–10. [PubMed: 18662216]
 21. McDermott MM, Ferrucci L, Liu K, Criqui MH, Greenland P, Green D, et al. D-Dimer and Inflammatory Markers as Predictors of Functional Decline in Men and Women with and without Peripheral Arterial Disease. *J Am Geriatr Soc*. 2005; 53:1688–96. [PubMed: 16181167]

22. Davignon J, Ganz P. Role of Endothelial Dysfunction in Atherosclerosis. *Circulation*. 2004; 109:III27–32. [PubMed: 15198963]
23. Barbato JE, Tzeng E. Nitric Oxide and Arterial Disease. *J Vasc Surg*. 2004; 40:187–93. [PubMed: 15218485]
24. Healy B. Endothelial Cell Dysfunction: An Emerging Endocrinopathy Linked to Coronary Disease. *J Am Coll Cardiol*. 1990; 16:357–8. [PubMed: 2197313]
25. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-Dependent Dilation in the Systemic Arteries of Asymptomatic Subjects Relates to Coronary Risk Factors and Their Interaction. *J Am Coll Cardiol*. 1994; 24:1468–74. [PubMed: 7930277]
26. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, et al. Cigarette Smoking Is Associated with Dose-Related and Potentially Reversible Impairment of Endothelium-Dependent Dilation in Healthy Young Adults. *Circulation*. 1993; 88:2149–55. [PubMed: 8222109]
27. Jarvisalo MJ, Raitakari M, Toikka JO, Putto-Laurila A, Rontu R, Laine S, et al. Endothelial Dysfunction and Increased Arterial Intima-Media Thickness in Children with Type 1 Diabetes. *Circulation*. 2004; 109:1750–5. [PubMed: 15023875]
28. Maldonado FJ, de Miralles JH, Aguilar EM, Gonzalez AF, Garcia JR, Garcia FA. Relationship between Noninvasively Measured Endothelial Function and Peripheral Arterial Disease. *Angiology*. 2009; 60:725–31. [PubMed: 19054793]
29. Schroeder S, Enderle MD, Ossen R, Meisner C, Baumbach A, Pfohl M, et al. Noninvasive Determination of Endothelium-Mediated Vasodilation as a Screening Test for Coronary Artery Disease: Pilot Study to Assess the Predictive Value in Comparison with Angina Pectoris, Exercise Electrocardiography, and Myocardial Perfusion Imaging. *Am Heart J*. 1999; 138:731–9. [PubMed: 10502220]
30. Neunteufl T, Heher S, Katzenschlager R, Wolfl G, Kostner K, Maurer G, et al. Late Prognostic Value of Flow-Mediated Dilation in the Brachial Artery of Patients with Chest Pain. *Am J Cardiol*. 2000; 86:207–10. [PubMed: 10913483]
31. Yataco AR, Corretti MC, Gardner AW, Womack CJ, Katzel LI. Endothelial Reactivity and Cardiac Risk Factors in Older Patients with Peripheral Arterial Disease. *Am J Cardiol*. 1999; 83:754–8. [PubMed: 10080432]
32. Gardner AW, Parker DE, Montgomery PS, Scott KJ, Blevins SM. Efficacy of Quantified Home-Based Exercise and Supervised Exercise in Patients with Intermittent Claudication: A Randomized Controlled Trial. *Circulation*. 2011; 123:491–8. [PubMed: 21262997]
33. Lohman, TC., Roche, AF., Marubini, E. Anthropometric Standardization Reference Manual. Champaign, IL: Human Kinetics Books; 1988. p. 39-70.
34. Hiatt WR, Marshall JA, Baxter J, Sandoval R, Hildebrandt W, Kahn LR, et al. Diagnostic Methods for Peripheral Arterial Disease in the San Luis Valley Diabetes Study. *J Clin Epidemiol*. 1990; 43:597–606. [PubMed: 2189949]
35. Balta S, Balta I, Demirkol S, Cakar M, Sarlak H, Kurt O. Subclinical Peripheral Arterial Disease and Ankle-Brachial Index. *Angiology*. 2013; 64:395–6. [PubMed: 23716726]
36. Balta S, Demirkol S, Yesil FG, Cakar M, Sarlak H, Celik T. Only Ankle-Brachial Index May Not Be an Accurate Information About the Prevalence of Peripheral Arterial Disease. *Angiology*. 2013; 64:481–2. [PubMed: 23539614]
37. Oates CP, Allen J, Stansby G. Beyond the Ankle-Brachial Pressure Index for the Diagnosis of Peripheral Arterial Disease--Time for a New Look at Photoplethysmography. *Angiology*. 2013; 64:492–3. [PubMed: 23990644]
38. Ro du H, Lee DY, Moon HJ, Kim JH, Lee KM, Kim SJ. Peripheral Arterial Disease Assessment with Photoplethysmography and Continuous-Wave Doppler Ultrasound in Addition to Ankle-Brachial Index May Loss Time and Funds. *Angiology*. 2013; 64:322. [PubMed: 23389093]
39. Sarlak H, Cakar M, Balta S, Arslan E, Demirkol S, Akhan M. Peripheral Arterial Disease Assessment with Photoplethysmography and Continuous-Wave Doppler Ultrasound in Addition to Ankle-Brachial Index May Loss Time and Funds. *Angiology*. 2013; 64:321. [PubMed: 23299169]
40. 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. [PubMed: 18572363]

41. Ungvari Z, Labinskyy N, Mukhopadhyay P, Pinto JT, Bagi Z, Ballabh P, et al. Resveratrol Attenuates Mitochondrial Oxidative Stress in Coronary Arterial Endothelial Cells. *Am J Physiol Heart Circ Physiol*. 2009; 297:H1876–81. [PubMed: 19749157]
42. Torres JL, Ridker PM. High Sensitivity C-Reactive Protein in Clinical Practice. *Am Heart Hosp J*. 2003; 1:207–11. [PubMed: 15785190]
43. Mohler ER III, Hiatt WR, Creager MA. Cholesterol Reduction with Atorvastatin Improves Walking Distance in Patients with Peripheral Arterial Disease. *Circulation*. 2003; 108:1481–6. [PubMed: 12952839]
44. Gardner AW, Alaupovic P, Parker DE, Montgomery PS, Esponda OL, Casanegra AI. Influence of Peripheral Artery Disease and Statin Therapy on Apolipoprotein Profiles. *Int J Vasc Med*. 2013; 2013:548764. [PubMed: 24102029]
45. Gardner AW, Bright BC, Ort KA, Montgomery PS. Dietary Intake of Participants with Peripheral Artery Disease and Claudication. *Angiology*. 2011; 62:270–5. [PubMed: 21406424]
46. King DE, Egan BM, Geesey ME. Relation of Dietary Fat and Fiber to Elevation of C-Reactive Protein. *Am J Cardiol*. 2003; 92:1335–9. [PubMed: 14636916]

Table 1

Clinical characteristics of 160 symptomatic patients with peripheral artery disease. Values are means (SD) and percentages.

Variables	Values
Age (years)	64 (10)
Weight (kg)	83.0 (18.8)
Body Mass Index (kg/m ²)	29.1 (6.2)
Ankle/Brachial Index	0.71 (0.24)
Claudication Onset Time (sec)	193 (160)
Peak Walking Time (sec)	403 (262)
Sex (% Men)	54
Race (% Caucasian)	53
Current Smoking (% yes)	41
Veterans (% yes)	37
Hypertension (% yes)	87
Medication Use (% yes)	83
Number of Medications (n)	1.9 (1.3)
Dyslipidemia (% yes)	91
Medication Use (%)	70
Number of Medications (n)	0.9 (0.8)
Diabetes (% yes)	45
Medication Use (%)	39
Number of Medications (n)	0.7 (1.0)
Abdominal Obesity (% yes)	55
Metabolic Syndrome (% yes)	81
Metabolic Syndrome Components (n)	3.6 (1.3)
Obesity (% yes)	42
Lower Extremity Revascularization (% yes)	38
Coronary Artery Disease (% yes)	30
Myocardial Infarction (% yes)	18
Cerebrovascular Disease (% yes)	17
Cerebrovascular Accident (% yes)	15
Chronic Kidney Disease (% yes)	25
Chronic Obstructive Pulmonary Disease (% yes)	28
Dyspnea (% yes)	59
High sensitivity C-Reactive Protein (mg/L)	6.54 (13.27)
Oxidized Low Density Lipoprotein (U/L)	72 (25)
Hydroxyl Radical Antioxidant Capacity (Arbitrary Units)	0.95 (0.22)
Vascular Cell Adhesion Molecule-1 (pg/ml)	2231 (648)
Intercellular Cell Adhesion Molecule-1 (pg/ml)	2201 (1612)
Endothelial Cell Apoptosis (Arbitrary Units)	1.04 (0.36)
Endothelial Cell ROS production (Arbitrary Units)	29.07 (10.32)

Variables	Values
Endothelial Cell NF- κ B activity (Arbitrary Units)	1.37 (0.69)

ROS = reactive oxygen species.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2

Measures of calf muscle hemoglobin oxygen saturation (StO₂) during treadmill evaluation in symptomatic patients with peripheral artery disease. Values are means (SD).

Variables	Values
StO ₂ at rest (% saturation)	51 (20)
Minimum exercise StO ₂ (% saturation)	16 (19)
Time to minimum exercise StO ₂ (sec)	204 (252)
Absolute Drop in StO ₂ during exercise (% saturation)	35 (20)
Percentage Drop in StO ₂ during exercise (%)	70 (29)
Average Rate of Decline in StO ₂ (% saturation/sec)	0.57 (0.75)
StO ₂ at Peak Walking Time (% saturation)	21 (21)
Recovery Half-Time of StO ₂ (sec)	155 (163)
Recovery Time of StO ₂ (sec)	231 (227)
Recovery Time to Maximal StO ₂ (sec)	669 (269)
Maximum recovery StO ₂ (% saturation)	79 (21)

Table 3

Associations among measures of exercise performance, microcirculation, and measures of circulating antioxidant capacity, inflammation, and cultured endothelial cells treated with sera in symptomatic patients with peripheral artery disease.

Variables	Claudication Onset Time	Peak Walking Time	Time to minimum exercise Calf StO ₂	Average Rate of Decline in Calf StO ₂
High sensitivity C-Reactive Protein (mg/L)	-0.11	<i>-0.16[*]</i>	-0.04	0.22 [†]
Oxidized Low Density Lipoprotein (U/L)	-0.01	0.04	0.02	-0.08
Hydroxyl Radical Antioxidant Capacity	0.10	<i>0.27[†]</i>	0.09	-0.19 [*]
Vascular Cell Adhesion Molecule-1	-0.14	<i>-0.20[*]</i>	-0.05	-0.07
Intercellular Cell Adhesion Molecule-1	-0.07	-0.10	0.03	0.01
Endothelial Cell Apoptosis	0.03	0.03	-0.05	0.14
Endothelial Cell ROS production	0.04	0.12	0.15	0.06
Endothelial Cell NF- κ B activity	-0.27 [†]	<i>-0.33[‡]</i>	-0.21 [*]	<i>0.17[*]</i>

Values are Pearson partial correlation coefficients or Spearman partial correlation coefficients (indicated in italicized print), controlled for age, sex, race, ankle-brachial index, current smoking, body mass index, and obesity. ROS = reactive oxygen species, StO₂ = hemoglobin oxygen saturation.

^{*}
p < 0.05,

[†]
p < 0.01,

[‡]
p < 0.001.

Table 4

Regression coefficient summary for independent variables included in multivariate regression models for exercise performance and microcirculation dependent variables in symptomatic patients with peripheral artery disease.

Dependent Variables	Predictors	Regression Coefficient	95% Confidence Interval	Partial R ²	P Value
Claudication Onset Time (sec)	Endothelial Cell NF-κB activity	-51.60	-92.07 to -11.13	0.0544	0.013
	Intercept	254.59	192.51 to 316.67		
Peak Walking Time (sec)	ABI	386.95	198.24 to 575.66	0.1395	< 0.001
	Age	5.42	0.99 to 9.84	0.0547	0.017
	HORAC	329.57	88.00 to 571.14	0.0670	0.008
	Endothelial Cell NFKB	-84.46	-151.86 to -17.07	0.0571	0.015
Time to minimum exercise StO ₂ (sec)	Intercept	-409.21	-792.72 to -25.71		
	Endothelial Cell NFKB	-67.49	-132.88 to -2.11	0.0370	0.043
	Calf Muscle StO ₂ at rest	3.04	0.86 to 5.23	0.0652	0.007
	Intercept	133.35	-17.95 to 284.65		
	BMI	-0.04	-0.06 to -0.02	0.0878	0.001
Average Rate of Decline in StO ₂ (%/sec)	HORAC	-0.72	-1.35 to -0.10	0.0440	0.024
	Intercept	2.43	1.56 to 3.30		

ABI = ankle-brachial index, BMI = body mass index, HORAC = hydroxyl radical antioxidant capacity, StO₂ = hemoglobin oxygen saturation.

Overall model results: Claudication onset time: R² = 0.0544, p = 0.013, Peak walking time: R² = 0.2505, p < 0.001, Time to minimum exercise calf muscle StO₂: R² = 0.0995, p = 0.003, Average rate of decline in calf muscle StO₂: R² = 0.1349, p < 0.001.