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# Association between gait characteristics and endothelial oxidative stress and inflammation in patients with symptomatic peripheral artery disease

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**Abstract** The aim of the study was to determine whether gait characteristics were associated with endothelial cell inflammation, oxidative stress, and apoptosis and with circulating biomarkers of inflammation and antioxidant capacity in older patients with symptomatic peripheral artery disease (PAD). Gait measurements of 231 symptomatic men and women with PAD were assessed during a 4-m walk test. Patients were further characterized on endothelial effects of circulating factors present in the sera using a cell culture-based bioassay on primary human arterial endothelial cells and on circulating inflammatory and vascular biomarkers. In a multivariate regression model for gait speed, the significant independent variables were age ($p < 0.001$), intercellular cell adhesion molecule-1 (ICAM-1) ($p < 0.001$), diabetes ($p = 0.003$), sex ($p = 0.003$), and history of cerebrovascular accidents ($p = 0.021$). In multivariate analyses for gait cadence, the significant independent predictors included high-sensitivity C-reactive protein (HsCRP) ($p < 0.001$), diabetes ($p = 0.001$), and hypertension ($p = 0.001$). In a multivariate regression model for gait stride length, the significant independent variables were HsCRP ($p < 0.001$), age ($p < 0.001$), ICAM-1 ($p < 0.001$), hypertension ($p = 0.002$), cellular reactive oxygen species production ($p = 0.007$), and sex ($p = 0.008$). Higher levels of circulating biomarkers of inflammation and endothelial cell oxidative stress were associated with slower gait speed, slower cadence, and shorter stride length in older symptomatic patients with PAD. Additionally, this profile of impaired gait was more evident in older patients, in women, and in those with diabetes, hypertension, and history of cerebrovascular accidents.

**Keywords** Claudication · Inflammation · Gait speed · Mobility · Oxidative stress

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## Introduction

Peripheral artery disease (PAD) is a highly prevalent (Fowkes et al. 2013; Hirsch et al. 2006; Norgren et al. 2007), costly (Hirsch et al. 2008; Jaff et al. 2010; Mahoney et al. 2010; Sachs et al. 2011), and deadly condition (Brass and Hiatt 2006; Criqui et al. 1992), particularly in those older than 60 years (Hirsch et al. 2006). Additionally, PAD results in poor patient-

perceived health-related quality of life, primarily due to ambulatory leg pain and dysfunction (Gardner et al. 1991). Consequently, older patients with PAD have impaired physical function (McDermott et al. 2001, 2004), low physical activity levels (McDermott et al. 2000; Sieminski and Gardner 1997), and faster rates of functional decline and mobility loss over time compared to those without PAD (McDermott et al. 2001, 2002, 2004).

In recent years, gait speed has received considerable attention as a prognostic marker of health-related outcomes in older adults, and is of clinical relevance because it can be feasibly assessed in busy clinical settings (Cummings et al. 2014). Slow gait speed is associated with increased risk of all-cause-mortality (Studenski et al. 2011), frailty and independence (Liu et al. 2016), impaired gait efficiency (Ko et al. 2015), and increased risk of disability (Liu et al. 2016; Stephan et al. 2015), hospitalization (Liu et al. 2016; Stephan et al. 2015), and placement in long-term care (Cawthon et al. 2009; Vermeulen et al. 2011). Slow gait speed, as defined by a velocity slower than 0.8 m/s, is highly prevalent in men and women above age 65. As gait slows even further, the prevalence of disability dramatically increases (Cummings et al. 2014). Patients with symptomatic PAD are at particularly high risk for slow gait due to the development of leg pain during ambulation, thereby placing them at even higher risk for morbidity and mortality. In fact, we have found that gait speed is slower in PAD patients than in controls even during pain-free ambulation (Gardner et al. 2001). One possible mechanism for slow gait speed in older patients with PAD is their chronically high levels of inflammation and oxidative stress, which leads to muscle breakdown and sarcopenia (Clegg et al. 2013). However, the relationship between gait speed and inflammation and oxidative stress has not been examined in older patients with symptomatic PAD.

The aim of the study was to determine whether gait speed and its components of cadence and stride length were associated with endothelial cell inflammation, oxidative stress, and apoptosis and with circulating biomarkers of inflammation and antioxidant capacity in older patients with symptomatic PAD. We hypothesized that slower gait speed is associated with greater endothelial cell inflammation, 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 (Gardner et al. 2011).

### 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 (Lohman et al. 1988), cardiovascular risk factors, co-morbid conditions, claudication history, ankle-brachial index (ABI), fasting blood chemistries to assess lipids, glucose, insulin, and homeostasis model assessment (HOMA), and a list of current medications were obtained.

From this medical screening, patients were coded on cardiovascular risk factors according to standard definitions. For co-morbid conditions, coronary artery disease was defined by having at least one of the following conditions: a history of coronary percutaneous transluminal angioplasty, coronary stents, coronary artery bypass graft, myocardial infarction, or symptoms of exertional angina. Cerebrovascular disease was defined by having one of the following conditions: a history of carotid stents, carotid endarterectomy, carotid surgery, stroke, or transient ischemic attacks (TIA). Cerebrovascular accident was defined as having either a history of stroke or TIA. Chronic kidney disease was determined using the four-variable modification of diet in renal disease equation, and was defined as having an

estimated glomerular filtration rate <60 ml/min per 1.73 $m^2$ (National Kidney Foundation 2002).

*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 (Gardner et al. 1991), and (c) an ABI ≤ 0.90 at rest (Hirsch et al. 2006) or ≤0.73 after exercise (Hiatt et al. 1990). 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 344 individuals were evaluated for eligibility, and 231 patients were deemed eligible for inclusion into the study.

## Measurements

*Gait speed, cadence, and stride length obtained from a 4-m walk test* Gait speed, cadence (stride rate), and stride length were measured from a 4-m walk test in a hallway (Guralnik et al. 1994). Patients performed this test twice at their usual walking pace, and the faster of the two walks was used in the analyses. All patients performed the 4-m walk test pain free, as this test is too short to elicit claudication. The test-retest intraclass reliability coefficient is $R = 0.96$ for the velocity to walk 4 m (Gardner et al. 2004).

*Claudication onset time and peak walking time obtained from a graded maximal treadmill test* Patients performed a graded treadmill test to determine study eligibility, and then repeated the test on a following visit within 1 week to obtain the primary outcome measures of claudication onset time (COT) and peak walking time (PWT) as previously described (Gardner et al. 1991, 2011). Using our procedures, the test-retest intraclass reliability coefficient is $R = 0.89$ for COT (4) and $R = 0.93$ for PWT (Gardner et al. 1991).

*Total walk distance obtained from a 6-min walk test* Patients performed an over-ground, 6-min walk test supervised by trained exercise technicians, as previously described from our laboratory (Montgomery and Gardner 1998). The total distance walked during the test was recorded. The test-retest intraclass reliability coefficient is $R = 0.94$ for total 6-min walking distance (Montgomery and Gardner 1998).

*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 (Gardner et al. 2014; Ungvari et al. 2009). 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 (Gardner et al. 2014). 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 h. Caspase activities using Caspase-Glo 3/7 assay kit (Promega, Madison, WI) were measured to assess apoptotic cell death, as previously reported (Gardner et al. 2014).

*Cellular ROS production* Hydrogen peroxide ($H_2O_2$) production in detector endothelial cells was measured fluorometrically by a Tecan Inifinite M200 (Tecan Group Ag, Mannedorf, Switzerland) using the Amplex Red/horseradish peroxidase assay (Life Technologies, Grand Island, NY) to determine cellular ROS induced by factors present in the sera (Gardner et al. 2014).

*Transient transfection, nuclear factor K-light-chain-enhancer of activated B cells reporter gene assay* Transcriptional activity of nuclear factor K-light-chain-enhancer of activated B cells (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 (Gardner et al. 2014). Transfections in endothelial cells were performed using the Amaxa Nucleofector technology (Amaxa, Gaithersburg, MD), as we have previously reported (Gardner et al. 2014).

*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 (Gardner et al. 2014).

*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 metallopeptidase 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 Millipore 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* Concentration of high-sensitivity C-reactive protein (HsCRP) was quantified from 300 μl 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 (Torres and Ridker 2003).

## Statistical analyses

Measurement variables were summarized in Tables 1 and 2 by reporting means and standard deviations or medians and interquartile ranges, and dichotomized variables were summarized by reporting percentages. Associations reported in Table 3 are Spearman partial

**Table 1** Clinical characteristics of symptomatic patients with peripheral artery disease

| Variables | Values |
|---|---|
| Age (years) | 65 (10) |
| Weight (kg) | 83.9 (18.4) |
| Body mass index (kg/m$^2$) | 29.7 (6.2) |
| Ankle-brachial index | 0.72 (0.24) |
| Claudication onset time (s) | 152 (172) |
| Peak walking time (s) | 372 (368) |
| 6-min walk distance (m) | 346 (96) |
| Gait speed (m/s) | 1.08 (0.22) |
| Gait cadence (strides/min) | 53.0 (6.3) |
| Gait stride length (m/stride) | 1.22 (0.27) |
| Sex (% men) | 49 |
| Race (% Caucasian) | 49 |
| Current smoking (% yes) | 37 |
| Veterans (% yes) | 34 |
| Hypertension (% yes) | 88 |
| Dyslipidemia (% yes) | 90 |
| Diabetes (% yes) | 43 |
| Abdominal obesity (% yes) | 56 |
| Metabolic syndrome (% yes) | 80 |
| Obesity (% yes) | 46 |
| Lower extremity revascularization (% yes) | 35 |
| Coronary artery disease (% yes) | 34 |
| Myocardial infarction (% yes) | 18 |
| Cerebrovascular disease (% yes) | 16 |
| Cerebrovascular accident (% yes) | 15 |
| Chronic kidney disease (% yes) | 24 |
| Chronic obstructive pulmonary disease (% yes) | 25 |
| Dyspnea (% yes) | 59 |

Values are means (SD) and percentages

**Table 2** Inflammatory and vascular biomarkers of symptomatic patients with peripheral artery disease. Values are medians (interquartile ranges)

| Variables | Values |
|---|---|
| Endothelial cell measures | |
| Apoptosis (AU) | 1.10 (0.36) |
| Cellular ROS production (AU) | 27.55 (8.98) |
| NF-κB activity (AU) | 1.18 (0.86) |
| Circulating measures | |
| High-sensitivity C-reactive protein (mg/l) | 3.37 (5.20) |
| Tumor necrosis factor alpha (pg/ml) | 52 (26) |
| Interleukin-6 (pg/ml) | 23 (11) |
| Oxidized low-density lipoprotein (U/l) | 69 (36) |
| Hydroxyl radical antioxidant capacity (AU) | 0.95 (0.25) |
| E selectin (pg/ml) | 40 (48) |
| Vascular cell adhesion molecule-1 (pg/ml) | 2113 (907) |
| Intercellular cell adhesion molecule-1 (pg/ml) | 1920 (1327) |
| Vascular endothelial growth factor-A (pg/ml) | 27 (31) |
| Leptin (pg/ml) | 2095 (3073) |
| Adiponectin (pg/ml) | 5540 (1473) |
| Homeostasis model assessment | 2.34 (3.19) |
| Plasminogen activator inhibitor-1 (ng/ml) | 715 (482) |
| Apolipoprotein B (ng/ml) | 63 (56) |
| Apolipoprotein CIII (ng/ml) | 1268 (864) |

*ROS* reactive oxygen species

correlation coefficients, controlled for age, sex, race, ABI, current smoking, body mass index, and obesity. A stepwise regression procedure with entry $p$ value set at 0.05 was used to obtain regression models of Table 4. All analyses were performed using the NCSS statistical package. Statistical significance was set at $p < 0.05$.

## Results

The clinical characteristics of the patients are shown in Table 1. The ABI was lower than normal, indicative of PAD, which resulted in ambulatory impairments, as measured by COT, PWT, and 6-min walk distance. Furthermore, there was a high prevalence in cardiovascular risk factors and co-morbid conditions, such as hypertension, dylipidemia, diabetes, abdominal obesity, metabolic syndrome, and smoking.

Measures of inflammatory and vascular biomarkers of the patients are shown in Table 2. The association between these biomarkers and measures of gait characteristics are shown in Table 3, adjusted for age, BMI, ABI, sex, race, current smoking, and obesity. Gait speed was negatively correlated with HsCRP ($p < 0.05$), VCAM-1 ($p < 0.05$), and HOMA ($p < 0.05$). Gait cadence was negatively correlated with HsCRP ($p < 0.05$) and positively associated with HORAC ($p < 0.05$). Gait stride length was negatively correlated with VCAM-1 ($p < 0.05$), HOMA ($p < 0.05$), and apolipoprotein CIII ($p < 0.05$).

Multivariate regression models to predict gait characteristics are shown in Table 4. For gait speed, the significant independent variables were age ($p < 0.001$), ICAM-1 ($p < 0.001$), diabetes ($p = 0.003$), sex ($p = 0.003$), and history of cerebrovascular accidents (i.e., stroke and/or TIA) ($p = 0.021$). For gait cadence, the significant independent predictors included HsCRP ($p < 0.001$), diabetes ($p = 0.001$), and hypertension ($p = 0.001$). For the gait stride length, the significant independent variables were HsCRP ($p < 0.001$), age ($p < 0.001$), ICAM-1 ($p < 0.001$), hypertension ($p = 0.002$), endothelial cellular ROS production ($p = 0.007$), and sex ($p = 0.008$). Although height was considered as a potential predictor variable, it did not enter the regression models for gait speed, cadence, or stride length.

## Discussion

The primary novel findings were that greater impairments in gait characteristics of symptomatic patients with PAD were evident with higher levels of circulating biomarkers of inflammation (HsCRP and ICAM-1) and endothelial cell ROS production. Furthermore, impaired gait characteristics were found in older patients, in women, and in those with diabetes, hypertension, and history of cerebrovascular accidents.

### Gait impairments are associated with higher inflammation and oxidative stress

A key finding which supports our hypothesis was that multivariate analyses identified circulating biomarkers of inflammation and endothelial cell ROS production as independent predictors of gait speed, gait cadence, and gait stride length. Higher ICAM-1 was associated with slower gait speed and shorter stride length, higher HsCRP was predictive of slower gait cadence and stride length, and higher endothelial cell ROS production was predictive of shorter stride length. It is interesting to note

**Table 3** Associations among measures of gait characteristics and measures of inflammatory and vascular biomarkers in symptomatic patients with peripheral artery disease

| Variables | Gait speed | Gait cadence | Gait stride length |
|---|---|---|---|
| Endothelial cell measures | | | |
| Apoptosis ($n$ = 166) | −0.02 | 0.03 | −0.01 |
| Cellular ROS production | −0.06 | 0.01 | 0.05 |
| NF-κB activity ($n$ = 157) | −0.12 | −0.10 | −0.05 |
| Circulating measures | | | |
| High-sensitivity C-reactive protein ($n$ = 231) | −0.19* | −0.19* | −0.07 |
| Tumor necrosis factor alpha ($n$ = 172) | −0.09 | −0.00 | −0.09 |
| Interleukin-6 ($n$ = 172) | −0.12 | −0.01 | −0.08 |
| Oxidized low-density lipoprotein ($n$ = 189) | 0.11 | 0.10 | 0.07 |
| Hydroxyl radical antioxidant rapacity ($n$ = 162) | 0.08 | 0.16* | 0.02 |
| E selectin ($n$ = 172) | −0.10 | −0.02 | −0.07 |
| Vascular cell adhesion molecule-1 ($n$ = 172) | −0.21* | −0.00 | −0.22* |
| Intercellular cell adhesion molecule-1 ($n$ = 172) | −0.14 | 0.05 | −0.12 |
| Vascular endothelial growth factor-A ($n$ = 172) | 0.13 | 0.10 | 0.10 |
| Leptin ($n$ = 172) | −0.00 | −0.03 | 0.07 |
| Adiponectin ($n$ = 172) | −0.07 | 0.10 | −0.12 |
| Homeostasis model assessment ($n$ = 200) | −0.19* | −0.08 | −0.14* |
| Apolipoprotein B ($n$ = 172) | 0.05 | −0.02 | 0.07 |
| Apolipoprotein CIII ($n$ = 172) | −0.11 | 0.02 | −0.16* |

Values are Spearman partial correlation coefficients, controlled for age, BMI, ABI, sex, race, current smoking, and obesity

*ROS* reactive oxygen species

*$p < 0.05$

that all three biomarkers were included in the model for stride length. Chronically elevated levels of inflammation and oxidative stress activate muscle breakdown and promote sarcopenia (Chen et al. 2014; Clegg et al. 2013; Gaczynska et al. 1994), which may lead to reduced lower extremity strength and shorter stride lengths. These findings suggest that chronic inflammation and oxidative stress may mediate a slower gait speed in older patients with PAD partially due to shorter stride lengths.

Our current results support an earlier investigation from our laboratory that found endothelial inflammation, as measured by endothelial cell NF-κB activity, was predictive of COT and PWT obtained during a standardized treadmill test (Gardner et al. 2015). Interestingly, circulating biomarkers of inflammation were not predictive of treadmill COT and PWT, (Gardner et al. 2015) whereas they were predictive of over-ground gait measures in the current study. This supports previous studies that found 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 impairments in 6-min walk distance, pace of walking four meters, and the short physical performance battery (McDermott et al. 2003, 2008). Collectively, these results suggest that endothelial cell and circulating biomarkers of inflammation are negatively associated with ambulation during both over-ground, self-paced tests and standardized treadmill tests in older symptomatic patients with PAD.

## Gait impairments are associated with age, sex, and cardiovascular risk factors

A second key observation was that multivariate analyses identified age, sex, diabetes, hypertension, and history of cerebrovascular accidents as independent predictors of gait speed, gait cadence, and gait stride length. Greater age and female sex were associated with slower gait speed and shorter stride length, diabetes was predictive of slower gait speed and slower cadence, hypertension was associated with slower cadence and shorter stride

**Table 4** Regression coefficient summary for independent variables included in multivariate regression models for gait characteristic dependent variables in symptomatic patients with peripheral artery disease

| Dependent variables | Predictors | Regression coefficient | 95 % confidence interval | Partial $R^2$ | *p* value |
|---|---|---|---|---|---|
| Gait speed (m/s) | Age | −0.0073 | −0.0104 to −0.0042 | 0.0993 | <0.001 |
| | ICAM-1 | −0.0001 | −0.0001 to 0.0000 | 0.0634 | <0.001 |
| | Diabetes | −0.0921 | −0.1517 to −0.0326 | 0.0431 | 0.003 |
| | Sex | 0.0891 | 0.0306 to 0.1475 | 0.0419 | 0.003 |
| | CVA | −0.0897 | −0.1658 to −0.0136 | 0.0250 | 0.021 |
| | Intercept | 1.6388 | 1.4206 to 1.8571 | | |
| Gait cadence (strides/min) | HsCRP | −0.2127 | −0.2735 to −0.1518 | 0.1632 | <0.001 |
| | Diabetes | −2.5438 | −3.9577 to −1.1299 | 0.0432 | 0.001 |
| | Hypertension | −3.7805 | −1.5482 to −6.0128 | 0.0383 | 0.001 |
| | Intercept | 51.5478 | 49.4195 to 53.6760 | | |
| Gait stride length (m/stride) | HsCRP | −0.0069 | −0.0041 to −0.0096 | 0.1219 | <0.001 |
| | Age | −0.0080 | −0.0118 to −0.0043 | 0.0873 | <0.001 |
| | ICAM-1 | −0.0001 | −0.0001 to 0.0000 | 0.0644 | <0.001 |
| | Hypertension | −0.2279 | −0.3676 to −0.0882 | 0.0510 | 0.002 |
| | Cellular ROS | −0.0045 | −0.0013 to −0.0077 | 0.0374 | 0.007 |
| | Sex | 0.1008 | 0.0267 to 0.1748 | 0.0355 | 0.008 |
| | Intercept | 1.8534 | 1.5787 to 2.1280 | | |

Overall model results: gait speed $R^2 = 0.2327$, $p < 0.001$; gait cadence $R^2 = 0.2319$, $p < 0.001$; gait stride length $R^2 = 0.3181$, $p < 0.001$

*CVA* cerebrovascular accidents, *ICAM-1* intercellular cell adhesion molecule-1, *HsCRP* high-sensitivity C-reactive protein, *ROS* reactive oxygen species

length, and a history of cerebrovascular accidents was predictive of slower gait speed.

These gait results obtained from a simple and easily performed 4-m walk test support prior work from our laboratory utilizing more lengthy treadmill and 6-min walk tests. We recently found that age was an independent predictor of PWT during a standardized treadmill test in patients with PAD (Gardner et al. 2015), and women have a shorter treadmill PWT and lower peak oxygen uptake (Gardner 2002; Gardner et al. 2010) than men and a shorter pain-free and total 6-min walk distance (Gardner et al. 2010). Furthermore, our results support a previous study that found diabetic patients with PAD have a slower gait speed than those with diabetes (Dolan et al. 2002) and a previous report from our laboratory that patients with PAD and metabolic syndrome, a condition that often precedes diabetes, had shorter lower COT and PWT during a standardized treadmill test (Gardner et al. 2006). The slower cadence and shorter stride length of patients with hypertension in the current study support our earlier finding that PAD patients with hypertension have impaired exercise performance, as measured by slow oxygen kinetics during a constant-load, submaximal treadmill test (Ritti-Dias et al. 2013). Finally, slower gait speed in patients with a history of cerebrovascular accidents is in agreement with the well-established impairments in gait seen in stroke patients (Macko et al. 2002).

## 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 similar relationships exist among gait characteristics and inflammation 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

Higher levels of circulating biomarkers of inflammation and endothelial cell oxidative stress were associated with slower gait speed, slower cadence, and shorter stride length in symptomatic patients with PAD. Additionally, this profile of impaired gait was more evident in older patients, in women, and in those with diabetes, hypertension, and history of cerebrovascular accidents. The clinical implication is that interventions designed to reduce circulating inflammatory biomarkers and endothelial cell oxidative stress, such as dietary intervention, antioxidant therapy, and exercise training, may improve the gait of symptomatic patients with PAD partially through reductions in inflammation and oxidative stress.

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