Gender and racial differences in endothelial oxidative stress and inflammation in patients with symptomatic peripheral artery disease

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Background: **We compared (1) cellular reactive oxygen species (ROS) production, inflammation, and apoptosis of cultured endothelial cells treated with sera and (2) circulating inflammatory measures, antioxidant capacity, vascular biomarkers, and calf muscle hemoglobin oxygen saturation (StO_2) in men and women with peripheral artery disease (PAD). A secondary aim was to compare exercise performance and daily ambulatory activity between men and women. We hypothesized that women would have more impaired endothelial cellular ROS, inflammation, and apoptosis than men as well as worse systemic inflammation, antioxidant capacity, vascular biomarkers, calf muscle StO_2, exercise performance, and daily ambulatory activity.**

Methods: **The 148 symptomatic men and women with PAD were characterized on the endothelial effects of circulating factors present in the sera by a cell culture-based bioassay on primary human arterial endothelial cells. Patients were further evaluated by circulating inflammatory and vascular biomarkers, physical examination and medical history, exercise performance, and calf muscle StO_2 during exercise, and ambulatory activity was monitored during 1 week.**

Results: **Cellular ROS production was higher in African American women than in men ($P = .021$), but there was no gender difference in white individuals ($P = .537$). Men and women were not significantly different on endothelial cell apoptosis ($P = .833$) and nuclear factor κB activity ($P = .465$). For circulating factors, additional gender differences were found when comparisons were made within each race. In African Americans, women had higher intercellular adhesion molecule 1 ($P = .022$) and leptin ($P < .001$); whereas in white individuals, women had higher matrix metallopeptidase 9 ($P = .047$), higher vascular cell adhesion molecule 1 ($P = .047$), and lower hepatocyte growth factor ($P = .046$). Overall, women had higher apolipoprotein CIII ($P = .035$), lower pain-free distance ($P = .048$) and total distance ($P < .001$) during the 6-minute walk test, shorter time for calf muscle StO_2 to reach the minimum value during exercise ($P = .027$), and slower average cadence ($P = .004$) during daily ambulation.**

Conclusions: **African American women with symptomatic PAD have a heightened oxidative status, likely resulting in increased endothelial oxidative stress, compared with men. Furthermore, women exhibit a more pronounced proinflammatory profile of circulating biomarkers as well as more limited peripheral microcirculation, exercise performance, and ambulatory activity than men do. The clinical significance is that women with symptomatic PAD are in greater need than men of clinical intervention to improve oxidative stress, inflammation, and microcirculation, which may in turn have a favorable impact on their lower exercise performance and daily activity. (J Vasc Surg 2015;61:1249-57.)**

Peripheral artery disease (PAD) is a highly prevalent condition[1] that increases in people aged 65 years and older.[2] PAD is associated with increased prevalence of coexisting diseases in other arterial beds.[2,3] Concomitant cardiovascular and cerebrovascular disease in patients with PAD[3] contributes to their elevated rates of cardiovascular mortality.[4,5] The cost associated with PAD averages $3.9 billion for total Medicare paid PAD-related care annually,[6] which is greater than or similar to the costs associated with cardiac dysrhythmias, congestive heart failure, and cerebrovascular disease. Patients with PAD have ambulatory dysfunction and leg pain,[7] impaired physical function,[8,9] lower physical activity levels,[10,11] and even worse health-related quality of life scores than in individuals with coronary artery disease and congestive heart failure.[12] Furthermore, patients with PAD have increased rates of

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functional decline and mobility loss compared with those without PAD.[8,9,13]

For decades, the impact of atherosclerotic diseases in women was not fully appreciated.[14] Women suffer the consequences of PAD at rates at least as high as those observed in men.[15] Despite the high societal cost of PAD, clinical research to evaluate gender-based differences in the presentation and pathogenesis of PAD in women is limited. Our recent studies were the first to suggest that women with symptomatic PAD have greater ambulatory dysfunction than men, even though their ankle-brachial index (ABI) is similar.[16] We found that compared with men, women have lower daily physical activity[16] and slower ambulatory cadence[17] in the community setting and shorter claudication onset time (COT) and shorter peak walking time (PWT) during standardized treadmill exercise in the laboratory setting.[16] Greater impairment in calf muscle hemoglobin oxygen saturation (StO_2) during treadmill exercise in women explained their shorter PWT,[18] suggesting that women have worse microcirculation during exercise than men do. In addition, we have observed that women have a lower small artery elasticity index than men do,[19] providing further support that women have impaired microvascular function. Although patients with PAD have worse endothelial function[20,21] and higher levels of inflammation[22] and oxidative stress[23] than those without PAD, it is not clear whether there is a gender-related difference in these measurements in patients with PAD.

This study was designed to test the hypotheses that gender differences exist in vascular inflammatory and oxidative status in patients with PAD. To test our hypotheses, we used a bioassay approach to assess the effects of circulating factors present in the sera on endothelial reactive oxygen species (ROS) production, inflammation, and apoptosis using cultured endothelial cells and compared circulating inflammatory and vascular biomarkers and antioxidant capacity as well as calf muscle StO_2 in men and women with PAD. A secondary aim was to compare exercise performance and daily ambulatory activity between men and women.

METHODS

Patients

Approval and informed consent. The Institutional Review Board at the University of Oklahoma Health Sciences Center 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 laboratories and vascular clinics from the University of Oklahoma Health Sciences Center and the Oklahoma City VA Medical Center referred patients for possible enrollment into an exercise rehabilitation program for treatment of leg pain secondary to PAD.[24] The data and analyses for this study were part of the baseline assessments obtained for the exercise study.

Medical screening through history and physical examination. Patients were evaluated in the morning at the Clinical Research Center at the University of Oklahoma Health Sciences Center. 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,[25] cardiovascular risk factors, comorbid conditions, claudication history, ABI, blood samples, and list of current medications were obtained.

Inclusion and exclusion criteria. Patients with PAD were included in this study if they met the following criteria: history of ambulatory leg pain; ambulatory leg pain confirmed by treadmill exercise[7]; and ABI $\leq$ 0.90 at rest[2] or $\leq$0.73 after exercise.[26] Patients were excluded for the following conditions: absence of PAD (ABI $>$ 0.90 at rest and ABI $>$ 0.73 after exercise); noncompressible vessels (ABI $>$ 1.40); asymptomatic PAD; use of medications indicated for the treatment of claudication (cilostazol or pentoxifylline) initiated within 3 months before investigation; exercise limited by other diseases or conditions; active cancer; end-stage renal disease, defined as stage 5 chronic kidney disease; and abnormal liver function. A series of 216 consecutive individuals were evaluated, with 148 patients eligible and 68 subjects ineligible to participate.

Measurements

Graded treadmill test: COT, PWT, and calf muscle StO_2. Patients performed a graded treadmill test to determine study eligibility and to obtain outcome measures.[7] The COT and PWT were measured and are highly reliable, as previously described.[7] Ankle systolic blood pressure was obtained from the more severely diseased lower extremity before and 1 minute after the treadmill test.[7,27]

Calf muscle StO_2 was measured with the treadmill test by a continuous-wave, near-infrared spectroscopy unit (InSpectra model 325; Hutchinson Technology, Inc, Hutchinson, Minn), an optical cable attached to a 25-mm probe, InSpectra software (version 2.0), and a dedicated laptop computer as previously described.[28]

Six-minute walk test. Patients performed a 6-minute walk test, and the pain-free and total walking distances were recorded.[29] These measures are highly reliable, as previously described.[29]

Ambulatory activity monitoring. Daily ambulatory activity was assessed during 7 consecutive days with a step activity monitor (StepWatch3; Orthoinnovations, Inc, Oklahoma City, Okla), as previously described.[30] The step activity monitor is highly accurate and reliable, as previously described.[30]

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.

Endothelial cell cultures. A cell culture-based bioassay approach with cultured primary human arterial endothelial cells was used to characterize the endothelial