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Original Research

Influence of diabetes on ambulation and inflammation in men and women with symptomatic peripheral artery disease



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ABSTRACT

Objective: To determine whether diabetes and sex were factors associated with ambulatory function, endothelial cell inflammation, oxidative stress, and apoptosis, and with circulating biomarkers of inflammation and antioxidant capacity in patients with peripheral artery disease (PAD) and claudication.

Materials/Methods: Ambulatory function of 180 symptomatic men and women with PAD was assessed during a graded maximal treadmill test, 6-minute walk test, and 4-meter 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.

Results: Men and women with diabetes had greater prevalence ($p = 0.007$ and $p = 0.015$, respectively) of coronary artery disease (CAD) than patients without diabetes. To assure that this difference did not influence planned comparisons, the data set was stratified on CAD. Diabetic men with CAD had a lower peak walking time (PWT) during the treadmill test and a slower 4-meter gait speed compared to non-diabetic men with CAD ($p < 0.05$). Diabetic women with CAD had a lower PWT compared to their non-diabetic counterparts ($p < 0.01$). Additionally, diabetic men with CAD had higher pigment epithelium-derived factor ($p < 0.05$) than their non-diabetic counterparts, and diabetic women with CAD had higher leptin ($p < 0.01$) and interleukin-8 levels ($p < 0.05$).

Conclusions: In patients with PAD, diabetic men and women with CAD had more severe claudication than their non-diabetic counterparts, as measured by shorter PWT, and the men had further ambulatory impairment manifested by slower 4-meter gait speed. Furthermore, the diabetic patients with CAD had elevations in interleukin-8, leptin, and PEDF.

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Introduction

PAD is a significant medical concern, as it is a highly prevalent [1], costly [2], disabling [3,4], and deadly condition [5]. Exercise therapy is a primary treatment for patients with PAD, as the COT,

PWT, and 6-minute walk distance increase following a program of exercise rehabilitation [6–9]. However, the response to a program of exercise rehabilitation is variable, as we recently found that diabetic women responded poorly to a program of exercise compared to other subgroups of patients despite no difference in exercise adherence [10].

The relatively poor exercise response in diabetic women with PAD may be due to several possible factors. We have previously found that women have greater impairment in ambulation [11] and vascular function [12] compared to men. Furthermore, we recently found that women have greater inflammation than men [13], and that inflammation and anti-oxidant capacity were predictors of COT, PWT, and calf muscle hemoglobin oxygen saturation during exercise [14].

Abbreviations: ABI, ankle/brachial index; COT, claudication onset time; HsCRP, high sensitivity C-reactive protein; NF- κ B, nuclear factor K-light-chain-enhancer of activated B cells; PAD, peripheral artery disease; PEDF, pigment epithelium-derived factor; PWT, peak walking time; ROS, reactive oxygen species

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In addition to sex differences in ambulation and inflammation, diabetes has been found to impair ambulation in several [15,16], but not in all studies [17]. Diabetes may differentially impact women and men with PAD, but little is known about the sex-specific effect of diabetes on ambulatory and inflammatory profiles in patients with symptomatic PAD.

The primary aim of the current study was to determine whether diabetes and sex were factors associated with ambulatory function, endothelial cell inflammation, oxidative stress, and apoptosis, and with circulating biomarkers of inflammation and antioxidant capacity in patients with PAD and claudication. We hypothesized that patients with diabetes have impaired ambulation, greater endothelial inflammation, cellular ROS production, and apoptosis, and worse circulating inflammatory biomarkers and antioxidant capacity than those without diabetes. Furthermore, we hypothesized that the negative impact of diabetes on these outcome measures are more prominent in women than in men.

Methods

Patients

Approval and informed consent

The institutional review board at the University of Oklahoma Health Sciences Center approved the procedures of this study. Written informed consent was obtained from each patient at the beginning of investigation.

Recruitment

Patients who were not currently exercising were recruited from vascular laboratories and vascular clinics from the University of Oklahoma Health Science Center for possible enrollment into an exercise rehabilitation program to treat leg pain secondary to PAD.

Baseline clinical characteristics obtained from a medical history and physical examination

Patients were evaluated in the morning at the Clinical Research Center, at the University of Oklahoma Health Science 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, cardiovascular risk factors, co-morbid conditions, claudication history, ABI, and a list of current medications were obtained. Following the medical history and physical examination, nursing personnel obtained blood samples, and exercise personnel performed the exercise tests. The nursing and exercise personnel were blinded to the results from the medical history and physical examination, including the diabetes status of the patients.

Inclusion and exclusion criteria

Patients with symptomatic 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 [3], and (c) an ABI ≤ 0.90 [18] at rest or ≤ 0.73 after exercise [19]. 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 three 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 268 individuals were evaluated for eligibility, and 180 patients were deemed eligible for inclusion into the study.

Patients were grouped according to their diabetes status and sex. Diabetes was confirmed through medical history and list of medications, or by a glucose value of greater than or equal to 126 mg/dl in those patients without history or medication for diabetes. All patients with diabetes had Type 2 diabetes.

Measurements

COT and PWT 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 one week to obtain the primary outcome measures of COT and PWT as previously described [3,6]. Using our procedures, the test-retest intraclass reliability coefficient is $R = 0.89$ for COT [3] and $R = 0.93$ for PWT [3].

Total walk distance obtained from a 6-minute walk test

Patients performed an over-ground, 6-minute walk test supervised by trained exercise technicians, as previously described from our laboratory [20]. The total distance walked during the test was recorded. The test-retest intraclass reliability coefficient is $R = 0.94$ for total 6-minute walking distance [20].

Gait speed obtained from a 4-meter walk test

Gait speed was measured from a 4-meter walk test in a hallway [21]. Patients performed this test twice at their usual walking pace, and the faster of the two walks was used in the analyses. The test-retest intraclass reliability coefficient is $R = 0.96$ for the velocity to walk four meters [22].

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 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 [23]. 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 [23]. 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 [23].

Cellular ROS production

Hydrogen peroxide production in detector endothelial cells was measured fluorometrically using the Amplex Red/horseradish peroxidase assay to determine cellular oxidative stress induced by factors present in the sera [23].

Transient transfection, NF- κ B reporter gene assay

Transcriptional activity of NF- κ B 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 [23]. Transfections in endothelial cells were performed using the Amaxa Nucleofector technology (Amaxa, Gaithersburg, MD), as we have previously reported [23].

Serum antioxidant capacity

Hydroxyl radical antioxidant capacity using the OxiSelect hydroxyl radical antioxidant capacity 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 [23].

Circulating inflammatory and vascular biomarkers

A Milliplex Human Adipokine Magnetic Bead Kit was used for determining tumor necrosis factor alpha, interleukin-6, interleukin-8, leptin, and insulin. A Milliplex Human Cardiovascular Disease Panel 1 Kit was used for E selectin and vascular cell adhesion molecule-1. These assays were performed according to manufacturer's protocols. Sample protein content was determined for normalization purposes by a spectrophotometric quantification method using BCA reagent (Pierce Chemical Co., Rockford, IL).

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 [24].

PEDF

Plasma PEDF was measured using an ELISA kit from Millipore (#CYT420, St. Charles, MO) as described previously [25]. Briefly, plasma samples were incubated with 8 mol/l urea (Sigma Aldrich) at room temperature for 1 h and diluted 1:400. Samples were then applied in duplicate onto an antibody-coated plate and incubated at 37 °C for 1 h. After extensive wash, the plate was incubated with biotinylated mouse anti-PEDF antibody, followed by incubation with streptavidin peroxidase conjugate. After addition of 3,3',5,5'-tetramethylbenzidine, the plate was read (450 nm) by a Wallac-Victor3 1420 microplate reader. Intra and inter-assay coefficient of variations were 5.6 and 9.0% respectively.

Statistical analyses

Preliminary analysis revealed that coronary artery disease (CAD) prevalence in diabetic men (53%) compared to prevalence in non-diabetic men (25%) was significant ($p = 0.007$). Similarly, the prevalence in diabetic women (39%) compared to prevalence in non-diabetic women (15%) was also significant ($p = 0.015$). To assure that this difference did not influence planned comparisons, the data set was partitioned into CAD positive and CAD negative strata. Within each stratum clinical characteristics were summarized for diabetic and non-diabetic patients within sex. Summary statistics for measurement variables were means and standard deviations and percent of patients with characteristics present for dichotomous variables. Differences between sexes for measurement variables were examined using a 2 sample *t*-test. Between-sex differences for dichotomous variables were examined using a 1 degree of freedom chi-square test or Fischer exact test. Within each of the four diabetic/sex groups, the distributions for both the physical performances and

other response variables displayed appreciable departure from the normal distribution. Therefore, these variables were summarized as medians and inter-quartile ranges. The Wilcoxon non-parametric procedure was used to examine difference in medians for diabetic and non-diabetic patients within each sex within each stratum. The Wilcoxon test and Spearman correlation yield the same *p*-value for a dichotomous classification and a response variable. Hence, the partial Spearman controlled for other variables may be used as a non-parametric analog for the parametric ANCOVA to adjust *p*-values for extraneous variables. The *p*-value for partial Spearman correlation controlled for any adjusting variables is reported as adjusted *p*-value for comparisons between diabetic groups. As a guard against over parameterization, adjustments were made only for extraneous variables exhibiting statistical significance between groups. Variables such as obesity, BMI, etc. which reflect well recognized characteristics associated with diabetics were not regarded as extraneous variables and no adjustment was made for them, lest the adjustment reduce the effect of diabetes. All computations were made with the NCSS 2004 computer package. Statistical significance was defined as $p < 0.05$.

Results

The clinical characteristics of the patients are shown in Table 1. Compared to men without diabetes, men with diabetes had higher prevalence of obesity ($p < 0.001$), abdominal obesity ($p < 0.001$), metabolic syndrome ($p < 0.05$), dyslipidemia ($p < 0.05$), and coronary artery disease ($p < 0.01$), and they had higher BMI ($p < 0.001$) and greater number of metabolic syndrome components ($p < 0.001$). Compared to women without diabetes, women with diabetes had higher prevalence of obesity ($p < 0.001$), abdominal obesity ($p < 0.001$), metabolic syndrome ($p < 0.001$), coronary artery disease ($p < 0.05$), and they had higher BMI ($p < 0.001$), greater number of metabolic syndrome components ($p < 0.001$), lower age ($p < 0.01$), and lower percentage of Caucasians ($p < 0.05$).

The exercise measurements of the patients are displayed in Table 2. On average, diabetic men with CAD had a lower median PWT of 234 seconds compared to non-diabetic men with CAD ($p < 0.05$), and diabetic women with CAD had a lower median PWT of 272 seconds compared to their non-diabetic counterparts ($p < 0.01$). Additionally, the diabetic men with CAD had a slower median 4-meter gait speed of 0.21 meters/second than non-diabetic men with CAD ($p < 0.05$). No other group differences ($p > 0.05$) were found in the men and women.

The vascular biomarkers of the patients are shown in Table 3. Diabetic men with CAD had higher median PEDF ($p < 0.05$) and insulin levels ($p < 0.05$) than their non-diabetic counterparts. Additionally, diabetic women with CAD had higher median leptin ($p < 0.01$) and interleukin-8 levels ($p < 0.05$) than non-diabetic women with CAD. No other group differences ($p > 0.05$) were found in the patients.

Discussion

One novel finding was that diabetic men with CAD had impaired ambulation compared to their non-diabetic counterparts, as measured by a lower PWT and a slower 4-meter gait speed. Similarly, the diabetic women with CAD had lower PWT than non-diabetic women with CAD. Another novel finding was that diabetic men with CAD had higher PEDF than non-diabetic men, and that diabetic women with CAD had greater inflammation than women free of diabetes, as measured by elevated interleukin-8 and leptin.

Table 1

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

Variables	Diabetic men (n = 36)	Non-diabetic men (n = 59)	Diabetic women (n = 39)	Non-diabetic women (n = 46)
Age (years)	66 (9)	67 (10)	61 (7) [†]	67 (12)
Body mass index (kg/m ²)	31.8 (6.0) [‡]	27.0 (4.4)	32.2 (5.7) [‡]	26.9 (6.7)
Rest ankle/brachial index	0.75 (0.25)	0.70 (0.20)	0.66 (0.27)	0.70 (0.23)
Post-exercise ankle/brachial index	0.45 (0.33)	0.40 (0.28)	0.41 (0.32)	0.47 (0.34)
Race (% Caucasian)	58	68	51 [†]	65
Current smoking (% yes)	36	34	36	47
Hypertension (% yes)	92	85	95	80
Medication use (% yes)	88	92	97	89
Number of medications (n)	2.8	2.1	2.3	2.2
Dyslipidemia (% yes)	92 [*]	73	85	85
Medication use (% yes)	88	81	91	82
Statin use (% yes)	79	77	73	69
Number of medications (n)	1.3	1.2	1.1	1.3
Other medications				
Aspirin use (% yes)	64	71	64	57
Plavix use (% yes)	25	19	33	15
Abdominal obesity (% yes)	69 [‡]	34	82 [‡]	39
Metabolic syndrome (% yes)	92 [*]	73	100 [‡]	65
Metabolic syndrome components (n)	4.4 [‡]	2.8	4.6 [‡]	2.7
Obesity (% yes)	64 [‡]	25	62 [†]	28
Lower extremity revascularization (% yes)	31	24	36	39
Coronary artery disease (% yes)	53 [†]	25	38 [*]	15
Cerebrovascular disease (% yes)	8	19	15	17
Chronic kidney disease (% yes)	29	26	21	28
Chronic obstructive pulmonary disease (% yes)	14	27	38	30
Dyspnea (% yes)	53	49	77	65
Arthritis (% yes)	58	54	59	72

* Significantly different than non-diabetic group (p < 0.05).

† p < 0.01.

‡ p < 0.001.

Diabetes and impaired ambulation

Men and women with diabetes and concomitant CAD walked 234 and 272 fewer seconds during the graded maximal treadmill test than those with diabetes, indicating that they reached maximal claudication at a much lower exercise capacity. For example, the diabetic men with CAD reached a median final work stage of 4% grade, while those without diabetes walked to 8% grade, and in women the respective values were 2% and 6%. These data indicate that diabetic patients with CAD have more severe claudication and greater ambulatory impairment than those who are free of diabetes. These observations support our previous report that patients with PAD and metabolic syndrome, a condition that often precedes diabe-

tes, had shorter mean distances to the onset and to maximal claudication pain, respectively [26].

The lower PWT during the treadmill test in patients with diabetes and CAD is also of interest because the lower exercise capacity may potentially limit their daily ambulatory activities that are above minimal exercise intensities. This is evident with the 4-meter walk test, as they had an 18% slower gait speed than men without diabetes, which supports a previous study that found that PAD patients with diabetes had slower 4-meter walking speed than patients without diabetes [15]. The slower gait speed over four meters in the current study would project to 75 fewer meters covered during a 6-minute walk test, which would represent a large and clinically meaningful difference between the groups [27]. In fact, the actual

Table 2

Exercise measures of patients with peripheral artery disease. Values are medians (interquartile ranges).

Variables	Diabetic men (n = 36)	Non-diabetic men (n = 59)	Diabetic women (n = 39)	Non-diabetic women (n = 46)
Claudication onset time (s)				
CAD	120 (145)	165 (219)	107 (71)	107 (101)
No CAD	120 (93)	160 (232)	121 (115)	118 (168)
Peak walking time (s)				
CAD	281 (206) [*]	515 (393)	192 (140) [†]	464 (343)
No CAD	389 (347)	449 (393)	294 (331)	268 (299)
6-minute walk distance (m)				
CAD	321 (117)	383 (110)	244 (172)	368 (140)
No CAD	410 (44)	417 (94)	335 (1910)	349 (133)
Gait speed (m/s)				
CAD	0.99 (0.16) [†]	1.20 (0.24)	0.95 (0.29)	0.99 (0.08)
No CAD	1.05 (0.30)	1.08 (0.29)	1.03 (0.35)	0.98 (0.26)

Data for the women were adjusted for age and race.

* Significantly different than non-diabetic group (p < 0.05).

† p < 0.01.

Table 3

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

Variables	Diabetic men (n = 36)	Non-diabetic men (n = 59)	Diabetic women (n = 39)	Non-diabetic women (n = 46)
Apoptosis (AU)				
CAD	1.03 (0.40)	1.05 (0.25)	0.83 (1.09)	1.08 (0.22)
No CAD	1.05 (0.32)	1.21 (0.21)	1.01 (0.47)	1.02 (0.51)
Cellular ROS production (AU)				
CAD	24.58 (9.34)	28.73 (11.77)	20.37 (8.22)	29.07 (14.28)
No CAD	28.19 (6.10)	26.15 (7.09)	28.22 (4.50)	25.12 (5.56)
NF-κB activity (AU)				
CAD	1.11 (0.91)	1.40 (0.62)	0.82 (1.38)	1.25 (1.04)
No CAD	1.43 (0.99)	0.98 (1.10)	0.88 (0.97)	1.46 (0.99)
High sensitivity C-reactive protein (mg/L)				
CAD	3.78 (3.99)	2.40 (5.20)	3.80 (6.00)	3.30 (3.56)
No CAD	3.30 (2.31)	3.55 (4.89)	6.70 (5.90)	2.80 (5.30)
Tumor necrosis factor alpha (pg/ml)				
CAD	44 (14)	64 (26)	57 (32)	48 (34)
No CAD	59 (41)	45 (27)	49 (28)	48 (31)
Interleukin-6 (pg/ml)				
CAD	21 (26)	23 (6)	26 (20)	23 (14)
No CAD	26 (23)	27 (14)	24 (16)	21 (10)
Interleukin-8 (pg/ml)				
CAD	87 (45)	90 (61)	119 (37)*	76 (31)
No CAD	99 (47)	85 (49)	93 (89)	95 (55)
E selectin (pg/ml)				
CAD	44 (28)	27 (27)	38 (22)	34 (19)
No CAD	51 (68)	36 (25)	72 (62)	32 (37)
Vascular cell adhesion molecule-1 (pg/ml)				
CAD	2173 (1023)	2110 (1804)	1967 (813)	2732 (1190)
No CAD	1894 (886)	2060 (695)	2133 (1084)	2099 (759)
Leptin (pg/ml)				
CAD	2025 (1870)	1079 (2123)	4202 (1601)†	719 (3443)
No CAD	1665 (1677)	755 (862)	3459 (5184)	1889 (1988)
Pigment epithelium-derived factor (ng/ml)				
CAD	7941 (2184)*	3168 (1809)	8561 (5861)	11770 (0)
No CAD	7046 (5903)	5835 (3047)	10456 (6374)	5880 (4350)
Hydroxyl radical antioxidant capacity (AU)				
CAD	0.86 (0.29)	0.93 (0.39)	0.94 (0.29)	0.98 (0.19)
No CAD	0.90 (0.29)	0.93 (0.22)	0.93 (0.18)	0.92 (0.29)
Insulin (μIU/ml)				
CAD	10.4 (6.6)*	7.2 (12.0)	10.0 (10.7)	6.7 (16.9)
No CAD	9.2 (10.9)	6.9 (6.9)	10.1 (9.4)	4.5 (8.2)

AU = arbitrary units, ROS = reactive oxygen species.

Data for the women were adjusted for age and race.

* Significantly different than non-diabetic group ($p < 0.05$).† $p < 0.01$.

mean 6-minute walk distance of the diabetic men and women with CAD were 62 and 124 meters shorter than their non-diabetic counterparts, but these trends did not reach statistical significance.

A final interesting observation was that the impact of diabetes on PWT and gait speed was found in those with concomitant CAD, but not in patients free of CAD, suggesting that diabetes and CAD work synergistically to impair ambulation. This finding supports a previous observation from our laboratory that patients with CAD and stable angina have worse ambulation than in those without CAD during a longer 6-minute walk test that requires endurance, a shorter 4-meter walk test that is representative of typical daily ambulation at a usual preferred pace, and in patient-perceived ability to walk at various distances and speeds, and to climb stairs [28]. It is not clear why the combination of having diabetes and CAD is a particularly troublesome combination for ambulation in patients with PAD, but it may be that diabetes has peripheral effects and complications that impair lower extremity functioning [15,16], and CAD has central effects that impair aerobic fitness [29,30] as well as peripheral effects that impair muscular strength, endurance, and motor unit recruitment [31,32].

Diabetes and inflammation

The primary findings related to the inflammatory profiles of the current study was that diabetic women with CAD had greater levels of interleukin-8 and leptin than women free of diabetes, and diabetic men with CAD had greater levels of PEDF than in their non-diabetic counterparts. We have recently found that women with PAD have higher inflammation than men with PAD, and that race was a factor in explaining the sex-related differences in inflammation [13]. Thus, the present investigation extends these findings by showing that diabetes, in the presence of CAD, is another factor contributing to elevated inflammation in symptomatic patients with PAD. The higher levels of these inflammatory biomarkers in diabetic patients with CAD may partially explain why diabetes is a strong risk factor for PAD [33], and why those with diabetes represent a subgroup of patients with PAD who are more susceptible to progression of atherosclerosis [34], and increased risk of coronary events [34]. A noteworthy finding was that diabetic women with CAD had nearly a 6-fold higher leptin level than non-diabetic women. High levels of leptin are indicative of leptin resistance, which

may be an interface between inflammation and metabolism in obesity-related cardiovascular disease [35]. From a physical function standpoint, higher inflammation associated with diabetes may increase the risk of impaired ambulatory ability, as higher inflammation is associated with skeletal muscle protein breakdown [36], lower muscle mass [37], lower strength [37], worse functional performance [38], greater apoptosis in skeletal myocytes [39], higher percentage of fat within the calf muscle [37], and a greater decline in function during longitudinal follow-up [40].

A final interesting observation is that PEDF was higher in diabetic men with CAD than in their non-diabetic counterparts. PEDF is a neurotrophic factor secreted by adipocytes and hepatocytes with a variety of functions, one of which includes potent angiogenic inhibition [41]. PEDF inhibits endothelial cell migration [41], reduces endothelial proliferation [42], increases apoptosis of endothelial cells [43], and downregulates vascular endothelial growth factor [44]. Thus, higher levels of PEDF in the men with diabetes may further impair the already compromised circulation in the lower extremities secondary to PAD.

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. An additional limitation is that we did not measure the duration of having diabetes or the hemoglobin A1c levels, and thus do not have an estimate of long-term influence and control of diabetes. However, we believe the patients with diabetes were well-controlled prior to entering the study because most were taking medications, and no patient had glucose values, indicating that they were metabolically out of control. Another limitation is that it is difficult to separate the effects of obesity from diabetes on the outcome measures because of the close connection between the two. All of the patients with diabetes were either overweight or obese, and thus adjusting for obesity-related variables is essentially adjusting for diabetes in these groups of patients with PAD. Finally, there are limitations associated with the design of the study. Significant differences in ambulation and inflammation between diabetic and non-diabetic groups within each sex do 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 the sample is racially mixed, and has a high prevalence of typical risk factors for PAD such as dyslipidemia, hypertension, obesity, and smoking.

Conclusion and clinical significance

In patients with PAD, diabetic men and women with CAD had more severe claudication than their non-diabetic counterparts, as measured by shorter PWT, and the men had further ambulatory impairment manifested by slower 4-meter gait speed. Furthermore, the diabetic patients with CAD had elevations in interleukin-8, leptin, and PEDF. The primary clinical implication is that diabetic patients with CAD represent a subgroup of PAD patients who have particularly poor ambulatory function, and therefore should be high priority for targeted exercise therapy and dietary intervention to improve both ambulation and inflammatory profiles.

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Author contributions

AWG wrote the manuscript and takes responsibility for the manuscript. DEP performed the statistical analyses. PSM recruited patients and collected clinical data, and AIC referred patients and assisted with data collection. DS, ZU, AC, SXZ, JJW, and WES provided laboratory data analyses. All authors reviewed, edited, and approved the manuscript.

Conflict of interest

The authors declare they have no conflicts of interest.

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