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Walking Disability in Patients with Peripheral Artery Disease is Associated with Arterial Endothelial Function

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Abstract

Objective—Patients with peripheral artery disease (PAD) have varying degrees of walking disability that does not completely correlate with ankle brachial index (ABI) or angiographic anatomy. We hypothesized that endothelial function (EF) is an independent predictor of symptom severity in PAD patients.

Methods—This was a cross-sectional study of PAD (N=100) patients presenting to a vascular surgery clinic. All patients received ABI testing and brachial artery flow-mediated, endothelium-dependent, vasodilation (FMD) to assess arterial EF. Symptom severity and walking disability reported by Rutherford category was based on the patient's self-report during clinic visit, recorded by the investigator-vascular surgeons. Demographic, biochemical and physiologic parameters were entered into regression equations to determine association with symptom severity.

Results—Mean age was 66 ± 8 and 43% had diabetes. Mean FMD was 7.4% indicating impaired EF. EF progressively declined as Rutherford category increased (p=0.01). Brachial artery FMD, ABI, systolic blood pressure, C-reactive protein, LDL, HDL, beta-blocker use and a history of diabetes or coronary artery disease (CAD) were all associated with Rutherford category (all p<0.05). After multivariable regression, EF (p<0.02) and ABI (p<0.0001) were independently associated with walking disability. When the cohort was restricted to claudicants (n=73), EF remained associated with walking disability after adjustment for other covariates (p=0.0001).

Conclusion—Symptom severity in PAD is multifactorial, reflecting both impaired hemodynamics and vascular dysfunction. This is the first report demonstrating that walking

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CONFLICTS OF INTEREST/DISCLOSURES
None

disability in PAD is associated with arterial EF. The mechanistic link underlying these observations remains to be defined.

INTRODUCTION

Close to one third of primary care patients over 70 years old develop peripheral artery disease (PAD).[1] It has recently been reported that PAD is a worldwide disease and its incidence has increased by nearly a quarter in the last decade[2]. An advanced stage of the disease is characterized by impaired ambulation, loss of functional capacity, pain, non-healing wounds and limb loss, conferring significant morbidity and mortality. The advanced age and disability of patients with PAD also make them a highly vulnerable population with regards to major cardiovascular events[3]. Furthermore, loss of ability to exercise can further contribute to a decline in cardiovascular fitness. Understanding factors involved in walking impairment is therefore critical and could point towards strategies aiming to target the specific pathophysiological mechanisms involved.

Mechanisms of walking impairment in PAD remain poorly understood, but are likely multifactorial and involve impaired hemodynamics, abnormal muscle characteristics, arterial stiffness, and inflammation.[4–17] Previous studies have demonstrated that the ankle-brachial index (ABI) and other measures of PAD often poorly correlate with symptoms[1819]. The mechanisms responsible for walking impairment may involve factors beyond reduced blood flow, such as arterial stiffness, inflammation, arteriogenesis, nerve impairment and muscle dysfunction. It is presently unclear whether endothelial dysfunction is related to walking impairment in PAD and the relationships between ABI, endothelial function, and PAD-related functional impairment. The goal of this study was to characterize these relationships in a prospective cohort of patients with PAD.

METHODS

Study Population and protocol

This cross-sectional study investigated the relationship between endothelial function (EF) and walking disability in PAD patients. The investigator-initiated protocol was approved by the University of California, San Francisco (UCSF) Committee on Human Research (CHR) and all patients gave informed consent. Patients referred to the outpatient vascular surgery clinic of San Francisco Veterans Affairs Medical Center (SFVAMC) for evaluation of PAD were recruited. Patients with PAD were enrolled if they had at least one of the following inclusion criteria: symptoms of PAD (claudication or critical limb ischemia - CLI) associated with an ankle-brachial index (ABI) < 0.9, toe pressures <70 mm Hg, or imaging confirming ≥50% stenosis in the lower extremity arteries. Patients without PAD, CAD, cerebrovascular disease (CVD), and an ABI >0.9 were enrolled as controls. Exclusion criteria included: significant renal, hepatic, or inflammatory disease, concurrent severe infections, acute illness or other major surgery within 30 days or taking immunosuppressive medications. We recorded demographic and anthropometric data, cardiovascular history, risk factors, concurrent medications, and pertinent cardiovascular examination findings. EF was measured by flow-mediated brachial artery vasodilation (FMD). Other measurements included hsCRP, lipid panel (LDL, triglycerides, HDL, total cholesterol), blood pressure and bilateral ABIs[20].

Measurements

Demographic and Anthropometric Data, Hemodynamic Measurements and Walking Distance—Demographic and anthropometric data collected included age, race, gender, hip and waist circumference, body mass index, prior supplement use, and exercise

frequency. We collected cardiovascular history, such as CAD, CVD, and previous procedures, as well as risk factors including hypertension, diabetes, hypercholesterolemia, cigarette smoking, and renal insufficiency. Concurrent medications and pertinent cardiovascular examination findings were also recorded. Blood pressure was measured by an indirect sphygmomanometer. Walking distance and Rutherford classification was based on the patient's self-report during clinic visit, recorded by the investigator-vascular surgeons (Table 1).

Vascular Reactivity of Brachial Arteries—Flow-mediated vasodilation was performed according to current guidelines and standards.[2122] Subjects were asked to fast (≥ 8 hours) and abstain from nicotine (≥ 4 hours) before the exam. The examination takes an average of 40 minutes and is performed by the research assistant under direct supervision by a vascular surgeon. A history of recent medications was recorded. Subjects were allowed to rest for ten minutes in a supine position in a darkened room at 23°C. The subject's arm was extended onto a movement-constraining pillow with the palmar aspect oriented anteriorly. A 5 cm tourniquet blood pressure cuff was placed on the upper arm proximal to the insertion of the deltoid. The length of the brachial artery was surveyed by B-mode ultrasound (Philips HD11) using a broadband linear array transducer with a 3–12 MHz range (Philips L12-3) until a straight segment with a visible registration structure can be located. The probe was oriented so that the artery is at least 3 cm deep to the surface of the skin, the focus aligned with the deep boundary of the vessel, and clearly demarcated intima/lumen boundaries were visible.

Prior to cuff inflation, the baseline diameter of the vessel was recorded for 60 seconds using EKG-gated image capture software (Brachial Imager, Medical Imaging Applications LLC, Coralville, IA). Baseline blood-flow velocity was recorded for 60 seconds using an insonation angle of 60°. The Doppler sample gate was positioned to cover the center, but not the edges, of the lumen. The probe was not moved between measurements.

The blood pressure cuff was inflated to the greater of 250 mm Hg or 50 mm Hg above the subject's systolic blood pressure for a period of 5 minutes. Recording of the B-mode images began 10 seconds prior to cuff release. Blood-flow velocity was assessed for a period of 30 seconds post-cuff release using the methods described above. B-mode images was recorded until 3 minutes post-cuff release.

Analysis of the images were performed using continuous edge-detection software (Brachial Analyzer, Medical Imaging Applications LLC, Coralville, IA). Baseline diameter was recorded as the mean of 60 seconds data. From hyperemia recordings, the exact moment of cuff release was noted. Hyperemia diameter was calculated using a pre-determined time window (55–65 seconds post-cuff release). FMD% was calculated as (60s Hyperemia diameter-Avg Baseline diameter/Avg Baseline diameter)*100.

Time averaged velocity measurements was obtained using the peak-velocity method. Average velocity at baseline was obtained from 60 seconds of data. Velocity of the hyperemia stimulus was calculated as the mean velocity of the first four heart beats following cuff-release. Both mean velocity and the velocity time integral were recorded.

Quality control was assessed at each point of the measurements. Image quality was evaluated by a 2nd person and graded on a 6 point scale that includes: registration structure (landmark), horizonally directed artery, correct longitudinal alignment, clearly visualized near wall intimal medial thickness (IMT) and far wall IMT, and at least 5 mm of clearly visualized artery. The interobserver variability in our laboratory is 0.05 ± 0.16% and the intraobserver variability is 0 ± 0.15%.

Ankle-Brachial Index—Ankle-brachial indices were measured using current guidelines and standards.[20] The procedure takes an average of 10 minutes. Systolic blood pressures of the brachial, posterior tibial and dorsalis pedis arteries were measured bilaterally. For each lower extremity, the highest systolic pressure of the two pedal pulses were divided by the highest systolic pressure of the two brachial arteries.

Renal, Lipid, Metabolic and Inflammatory Measurements—Blood samples were collected in a fasting state for measurement of creatinine (Cr), estimated glomerular filtration rate (eGFR), albumin, hemoglobin A1C (Hgb A1C) if diabetic as well as total cholesterol, triglycerides, low-density lipoprotein and high-density lipoprotein. Plasma was assayed for these analytes the same day as collection by the SFVAMC lab per standard methodology (Beckman Coulter Analyzer). Serum was isolated at the same time points for homocysteine and assayed the same day as collection by the SFVAMC lab per standard methodology (Abbott Diagnostics Architect i1000 Analyzer, Lake Forest, IL). The inflammatory marker hsCRP was measured from plasma assayed the same day per standard methodology (Beckman Coulter Analyzer, Miami, FL). The coefficient of variation for hsCRP using this procedure is 5.1%.

Statistical Analysis

For descriptive purposes, we categorized participants *a priori* by Rutherford category. They were then further grouped by PAD category for the overall analysis (no PAD –Rutherford 0, claudicants – Rutherford 1 to 3, and CLI – Rutherford 4 to 6). Differences in baseline characteristics were compared with the use of ANOVA for continuous variables and the chi-squared test for dichotomous variables. Since hsCRP had a skewed distribution, it was log-transformed for statistical analyses. For the overall regression models, patients were divided based on the PAD category (no PAD- Rutherford 0, claudicants –Rutherford 1 to 3, and CLI – Rutherford 4 to 6). For regression models in claudicants, the Rutherford category was used as a categorical variable. We used multivariable linear regression models to determine the relationship, expressed as adjusted means by category, between the PAD category or Rutherford category and FMD. Multivariate adjustment was made for demographic characteristics as well as covariates known to influence the Rutherford category based on an *a priori* determination of significance at $p<0.05$ on univariate models. Models were then repeated to assess the relationship between the ABI and symptomatology of patients. Statistical analyses were performed using Stata/SE 12 (StataCorp, College Station, TX).

RESULTS

A total of 100 patients participated in this study (73 with claudication, 19 with CLI and 8 without PAD). Table 2 demonstrates the demographics, comorbidities, medications, and PAD risk factors associated with all participants as well as with each of these groups. The mean age was 66 ± 8, 91% had hypertension, 87% had diagnosed hyperlipidemia, 39% had a history of CAD, and 43% had diabetes mellitus. Increasing Rutherford category was associated with a lower FMD, lower ABI, higher systolic blood pressure, and a higher incidence of diabetes mellitus and CAD as well as higher CRP and lower albumin (Table 2 and Figures 1 and 2).

In assessing factors predicting the Rutherford category symptomatology of patients using a univariate analysis, the factors with strongest association included FMD ($p <0.0001$), ABI ($p<0.0001$), beta-blocker use ($p<0.0001$), LDL ($p=0.001$), HDL ($p=0.006$), CRP ($p=0.007$), a history of CAD ($p=0.002$) or diabetes mellitus ($p=0.006$), and SBP ($p=0.01$). After adjustment for these factors, FMD was still significantly associated with Rutherford category (Table 3) with an adjusted mean FMD of 11.4% among controls, 8.0% among

claudicants and 5.3% in CLI patients (p=0.02). Within the same cohort, after adjustment for factors associated with walking impairment, the ABI also remained significantly associated with Rutherford category (Table 3) while none of the other factors remained associated with Rutherford category.

When the cohort was restricted to claudicants (n=73), brachial artery FMD decreased with worsening Rutherford category (Table 4). A strong association was also found between FMD and walking impairment (p=0.0001) (Table 5). After adjustment for age, race, SBP, index ABI, LDL, HDL, CRP, diabetes, history of CAD, and beta-blocker use, FMD remained significantly associated with walking impairment with an adjusted mean FMD of 11.4% among patients with Rutherford category 1, 9.3% with Rutherford category 2 and 6.6% with Rutherford category 3 symptoms (p=0.0008) (Table 5). The ABI did not hold a significant independent association with walking impairment in claudicants, nor did the other factors (Table 5).

FMD and index ABI were not correlated in the entire cohort (p=0.18), indicating that FMD independently predicts symptom severity.

DISCUSSION

In a cross-sectional cohort study of patients presenting to our outpatient vascular surgery clinic, we found a significant association between EF as measured by brachial artery FMD and disease severity in patients with PAD. Since the ABI and FMD were not correlated, we conclude that endothelial dysfunction is associated with walking disability independently of ABI. However the mechanisms underlying this association remain to be elucidated.

Multifactorial Etiology of Walking Disability in Patients with PAD

Walking disability in patients with PAD is likely multi-factorial and involves abnormal muscle characteristics, inflammation, impaired hemodynamics, and arterial dysfunction. In addition to the inadequacy of arterial blood flow and collateralization, many tissue analyses and animal studies have shown that overall adverse calf muscle characteristics[6] can be attributed to impaired calf muscle mitochondrial function,[5] impaired calf muscle function and metabolism,[5–7] reduced tissue perfusion, and lean muscle atrophy.[4] Increased calf muscle proteolysis coupled with inflammation[8–10] also plays a key role in walking disability. CRP is known to lead to release of endothelial monocyte chemoattractant protein-1 that attracts monocytes to the endothelium, up-regulation of tissue factor and pro-inflammatory cytokines such as TNF-α, inhibition of nitric oxide, and induction of endothelial adhesion molecules such as sICAM-1 and sVCAM-1 leading to adhesion of monocytes to the endothelium.[19–21] McDermott et al. previously demonstrated that inflammatory markers including CRP, IL-6, ICAM-1 and VCAM-1 are associated with shorter walking distance and slower walking speeds.[19–21]

To our knowledge, this report is the first to examine the relationship between walking disability and EF evaluated by brachial artery FMD in a PAD cohort. Several studies have found that endothelial dysfunction and reduced endothelium-mediated vasoreactivity are associated with PAD severity[2324]. Coutinho et al.[16] explored the philosophy behind the potential influence of EF in the functional decline of PAD patients. Various clinical trials followed and showed that greater physical activity and exercise can enhance EF as measured by FMD[25–27]. We therefore confirm through this cross-sectional study that EF assessed by brachial artery FMD could serve as a risk marker for symptom severity and impaired physical activity in patients with PAD. There is still controversial evidence over the effect of the nitric oxide-mediated vasodilation mechanism and whether or not nitric oxide supplementation can improve claudication distance and exercise tolerance in PAD

patients[28,29]. Thus further studies are warranted to delve into the physiological relationship between EF and walking disability as well as therapeutic strategies.

Arterial function, the ABI and Endothelial function

Arterial function can be described by arterial stiffness and EF. Arterial stiffness measured by pulse-wave analysis and velocity and augmentation index has been associated with greater functional impairment.[11] Impaired hemodynamics and degree of arterial stenosis as measured by ABI should intuitively influence walking disability as well. The ABI is a simple and quick method of detecting PAD that is also office-based, noninvasive, inexpensive, and easily reproducible[20]. Nevertheless, a meta-analysis reviewing 33 clinical studies with 1237 PAD patients showed that clinical improvements were not entirely correlated with ABI[30].

Although numerous epidemiological studies have demonstrated that the ABI is an independent predictor of mortality,[31–34] its relationship to claudication symptoms is not entirely clear. Furthermore, its relationship to EF is not fully understood. For example, in a study of PAD patients, a low ABI was found to be independently related to a low FMD.[35] In another study assessing PAD severity and inflammation, FMD was found to correlate with ABI.[36] Still, other studies have found no correlations between the ABI and FMD[37]. In our patient population, EF and ABI were both associated with walking impairment, although the two of them were not directly correlated. This suggests that they are both factors that could independently influence symptomatology of patients with PAD.

While our findings indicate that decreasing ABI was independently associated with patient-reported claudication symptoms, the measurement of ABI at rest may not be the best reflection of PAD severity. Patients found to have a normal ABI (> 0.9) may still have significant leg pain at rest due to mild disease or arterial occlusive symptoms, which can produce a falsely-negative ABI reading. Exercise testing with pre- and post-ABI assessment, rather than resting ABI, has been shown to be a more sensitive screening tool for these patients[38–40]. Exercise, which can include a graded treadmill testing, a 6-minute walk test, active pedal plantar flexion,[40] or even an arm-leg ergometry,[41] affects flow across a moderately stenotic vessel and exposes a lower ABI compared to rest. If the exercise ABI readings are normal, then leg pain is likely not associated with PAD and can suggest a neurogenic cause or muscle pathology. If the post-exercise ABI measurements are abnormal, then PAD is more likely.

Beta-blockade and Claudication Symptom Severity—Patients with PAD have, by definition, systemic atherosclerosis and are highly likely to be affected by CAD as well. A number of lifestyle factors are known to contribute to the progression of atherosclerosis and development of CAD and PAD, the most significant being hypertension, smoking, dyslipidemia, poor glycemic control, and increased levels of circulating inflammatory biomarkers. Beta-blockade medications are among the most common medical therapy prescribed to patients with PAD, and they serve mainly to mitigate the detrimental effects of uncontrolled hypertension. To this point, 61% of patients in our cohort were taking a beta-blocker. We interpret this high proportion to mean that use of this medication is a strong surrogate for atherosclerotic disease burden. Atherosclerotic plaque deposition is associated with stiffer and more stenotic vessels, weakened EF and reduced vasoreactivity. Our findings indicate that such impairments in vascular function have important clinical significance, namely in reducing walking capacity. Despite optimal medical therapy aimed at known risk factors (along with beta-blockers, this includes additional anti-hypertensive medications, statins and anti-platelet agents), many patients do not experience clinical improvement. In fact, in our cohort frequency of beta-blocker use increased with worsening

symptomatology: 22%, 42% and 71% of patients across Grades I, II and III, respectively. This raises the important concern that current available medications, while helpful at reducing cardiovascular risk, are not enough and there is a need to identify additional therapies that augment the endothelial dysfunction associated with PAD.

Interventions to Improve Endothelial Function—One such promising therapy is Ramipril, an angiotensin-converting enzyme inhibitor (ACE-i), that was recently shown to improve exercise capacity and enhance quality of life in patients with symptomatic PAD. In a 24-week trial, there was an average 77% improvement in pain-free walking and a 123% gain in maximal walking time, corresponding to 75 sec and 255 sec increases, respectively.[42] The authors proposed that ACE-inhibition with Ramipril may induce vasodilation by way of reduction in angiotensin-II and also improve peripheral blood flow and endothelial function due to bradykinin preservation, thus leading to better functioning. These findings are especially exciting when comparing to cilostazol and pentoxyfilline, the only medications currently approved by the US Food and Drug Administration for treatment of claudication associated with PAD, with cilostazol conferring a greater symptomatic benefit that approaches 25%.[43]

Another emerging intervention that may lead to improvement in EF is supplementation with n-3 polyunsaturated fatty acids (n-3 PUFAs). In one notable study of young, healthy smokers, Siasos et al found that FMD values significantly improved after oral treatment with 2gm/day of n-3 PUFAs at various time intervals spanning several months[44]. The reasoning underlying this correlation is that fatty acids may improve EF by decreasing the elevated oxidative stress caused by smoking. N-3 PUFAs supplementation could lead to recovery of endothelial synthesis of nitric oxide and PGI2, as well as vascular smooth muscle cell sensitivity to NO. These mechanisms are especially relevant to the cohort evaluated in our study, 94% (n=94) of whom are current or were past smokers and who, as a result, have pro-inflammatory profiles.

Exercise can also be prescribed as an effective therapy for patients with claudication. Beneficial effects of exercise may include increasing collateral flow, improving EF through increased NO-dependent vasodilation and thereby improving ABI, augmenting mitochondrial energy production and decreasing circulating inflammatory molecules.[45] Exercise therapy, therefore has the potential to reverse the pathologic mechanisms associated with PAD and interrupt progression toward further disability. One study testing a 6-month exercise rehabilitation intervention in symptomatic PAD patients and controls found significant improvements in treadmill times to onset and maximal claudication pain, as well as ABI.[46] A randomized study of 156 PAD patients by McDermott and colleagues[47] found that 6 months of treadmill exercise led to increase in FMD, implying improvement in EF, and symptom improvement: exercising patients' 6-minute walk distance increased by 20.9m compared with a decline of 15m among non-exercising controls. Post-exercise ABI was not reported, though in theory, improvement could be expected in the setting of improved hemodynamics. Another randomized controlled trial of 104 patients with PAD and intermittent claudication showed that performing either arm or leg exercises, as compared to no therapy among controls, improved time to onset of claudication and maximal walking distance at 6, 12, 18, and 24 weeks. Progressive improvements at each time interval were also observed.[48] These results emphasize one of the primary goals in managing patients with PAD— to improve disease-related impairment.

Limitations

The patient population studied was not representative of the wider PAD population as it included only male veterans from SF VAMC referred to a vascular surgery clinic hence the

findings do not extend to women. It is important to state that this paper does not address the majority of patients with PAD- those who are asymptomatic. Another major limitation of the study is that the reported Rutherford classification was based on self-report by patients and not verified by walking impairment questionnaire. Furthermore, there was no direct functional testing using a treadmill or 6-minute walk test. In order to address this limitation, the walking impairment questionnaire and 6-minute walk test have been added to upcoming studies at our institution. Another limitation of this study is that the controls were controls for PAD though they may have had occult CAD or CVD. Furthermore, this report does not imply causation but rather an association. Lastly, although it is known that cigarette smoking chronically[49] and acutely[50] alters EF, it is less likely that it was a factor in the present study as there was no difference in the baseline FMD of smokers vs non-smokers. This is likely related to the very severe atherosclerotic burden and disease severity of our patient population, i.e. veterans with PAD.

CONCLUSIONS

In a contemporary cohort of patients, vascular function as measured with brachial artery FMD is associated with symptom severity in patients with PAD, independently of the ABI. This supports the premise that symptom severity in PAD is multifactorial, adding vascular dysfunction to other important factors including muscle characteristics, inflammation, and impaired hemodynamics. Although the mechanisms remain unclear, our data suggest the possibility that interventions that improve EF could have a positive impact on symptomatology in patients with PAD.

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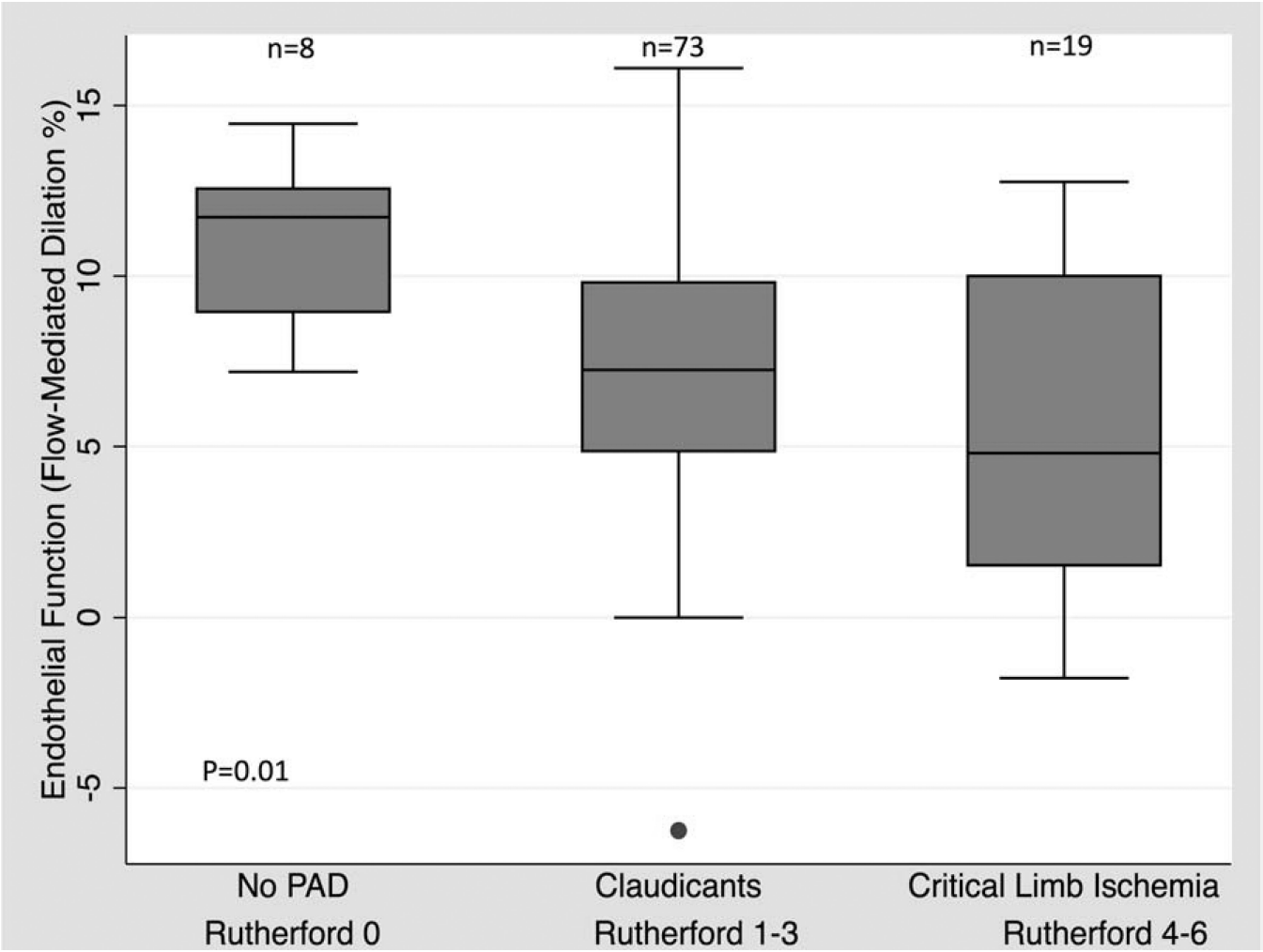

Figure 1. Relationship between symptomatic status of patients and Brachial FMD

Brachial artery flow-mediated vasodilation by PAD category in the entire cohort, unadjusted data. P-value for difference between groups using ANOVA.

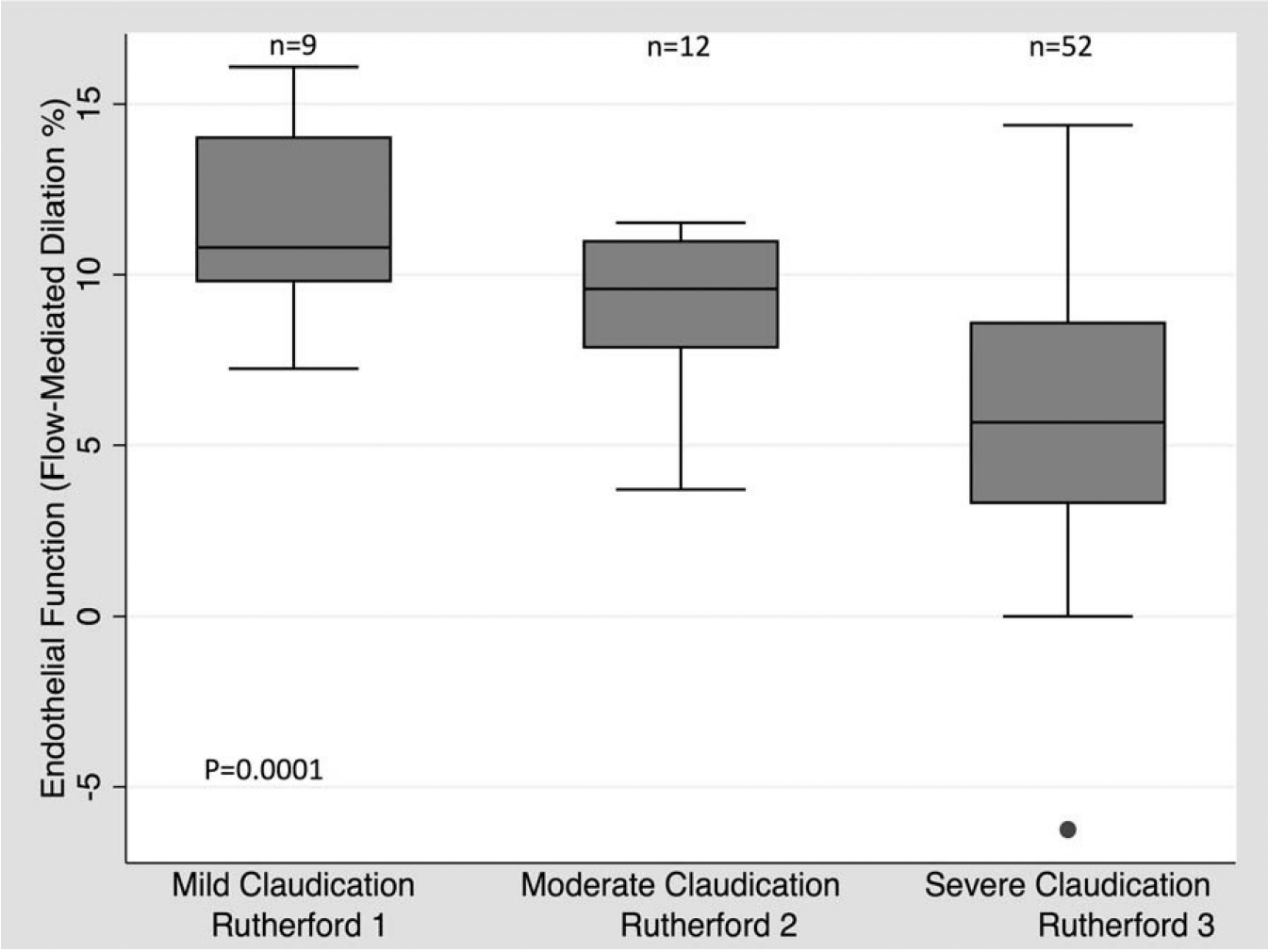

Figure 2. Relationship between symptomatic status of claudicants and Brachial FMD

Mean brachial artery flow-mediated vasodilation by Rutherford category in patients with claudication, unadjusted data. P-value for difference between groups using ANOVA.

Table 1

Rutherford Category used in the study.

Grade	Category	Clinical	Distance
0	0	Asymptomatic	
I	1	Mild claudication	>3 blocks
I	2	Moderate claudication	>1 and 3 ≤ blocks
I	3	Severe claudication	≤1 block
II	4	Ischemic rest pain	
III	5	Minor tissue loss	
III	6	Major tissue loss	

TABLE 2

The baseline characteristics of the population categorized by PAD category

Characteristics	All patients (n=100)	No PAD Rutherford 0 (n=8)	Claudicants Rutherford 1–3 (n=73)	Critical Limb Ischemia Rutherford 4–6 (n=19)	P-value
Age, Mean (SD), y	66 ± 8	63 ± 8	67 ± 8	67 ± 9	0.47
Male Sex (%)	100 (100)	8 (100)	73 (100)	19 (100)	-
Caucasian	67 (67)	3 (38)	53 (73)	11 (58)	0.09
BMI	28 ± 5	29 ± 3	28 ± 5	26 ± 6	0.47
Waist-hip ratio (%)	1.0 ± 0.1	0.98 ± 0.07	1.01 ± 0.06	1.0 ± 0.3	0.11
Systolic Blood Pressure (mmHg)	139 ± 22	132 ± 22	136 ± 18	152 ± 29	0.008
Diastolic Blood Pressure (mmHg)	75 ± 10	77 ± 8	75 ± 9	76 ± 13	0.74
Index ABI	0.68 ± 0.2	1.04 ± 0.24	0.69 ± 0.15	0.50 ± 0.15	<0.0001
Brachial FMD (%)	7 ± 4	11 ± 3	7 ± 4	6 ± 5	0.01
Comorbidities					
Hypertension	91 (91)	6 (75)	67 (92)	18 (95)	0.24
Hyperlipidemia	87 (87)	6 (75)	66 (90)	15 (79)	0.24
Hx of CAD	39 (39)	1 (13)	26 (36)	12 (63)	0.03
Diabetes Mellitus	43 (43)	0 (0)	31 (42)	12 (63)	0.01
Medications					
Aspirin	66 (66)	3 (38)	47 (64)	16 (84)	0.06
Ace-inhibitor	42 (42)	1 (13)	34 (47)	7 (37)	0.16
B-Blocker	61 (61)	2 (25)	44 (60)	15 (79)	0.03
Statin	85 (85)	5 (63)	65 (89)	15 (79)	0.10
Insulin	22 (22)	0 (0)	13 (18)	9 (47)	0.006
PAD Risk Factors					
History of smoking	94 (94)	7 (88)	69 (95)	18 (95)	0.72
Total Cholesterol (mg/dl)	153 ± 42	186 ± 41	154 ± 42	134 ± 37	0.01
LDL (mg/dl)	83 ± 38	116 ± 43	82 ± 36	70 ± 33	0.01
HDL (mg/dL)	41 ± 13	44 ± 5	43 ± 13	36 ± 13	0.09
Triglycerides (mg/dL)	151 ± 92	129 ± 53	154 ± 97	149 ± 87	0.77
Serum creatinine (mg/dL)	1.1 ± 0.3	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.4	0.63

Characteristics	All patients (n=100)	No PAD Rutherford 0 (n=8)	Claudicants Rutherford 1–3 (n=73)	Critical Limb Ischemia Rutherford 4–6 (n=19)	P-value
Homocysteine	13.3 ± 4.8	10.1 ± 2.5	13.7 ± 4.9	13.2 ± 4.8	0.13
CRP (mg/L)	10.2 ± 25.7	4.1 ± 2.7	6.8 ± 19.8	25.2 ± 41.1	0.02
eGFR	79 ± 25	83 ± 19	78 ± 23	80 ± 31	0.87
Albumin	3.9 ± 0.4	4.1 ± 0.1	3.9 ± 0.4	3.6 ± 0.4	0.004

Values as “mean +/− SD” or “n (%)”.

TABLE 3

Adjusted means of brachial artery FMD and adjusted ABI by PAD category in the entire cohort.

Brachial Artery FMD							
Model	**Adjusted Mean Controls (Rutherford 0)**	**95% CI**	**Adjusted Mean Claudicants (Rutherford 1–3)**	**95% CI**	**Adjusted Mean CLI (Rutherford 4–6)**	**95% CI**	**p-value**
Model 1	11.0%	8.3, 13.7	7.4%	6.5, 8.3	5.8%	4.0, 7.7	0.01
Model 2	10.6%	7.8, 13.4	7.4%	6.5, 8.4	5.8%	4.0, 7.6	0.02
Model 3	11.4%	8.2, 14.6	8.0%	7.0, 8.9	5.3%	3.2, 7.5	0.02

Ankle-Brachial Index							
Model	**Adjusted Mean Controls (Rutherford 0)**	**95% CI**	**Adjusted Mean Claudicants (Rutherford 1–3)**	**95% CI**	**Adjusted Mean CLI (Rutherford 4–6)**	**95% CI**	**p-value**
Model 4	1.04	0.93, 1.15	0.69	0.65, 0.72	0.50	0.42, 0.57	<0.0001
Model 5	1.05	0.93, 1.16	0.68	0.65, 0.72	0.50	0.43, 0.58	<0.0001
Model 6	0.99	0.87, 1.11	0.69	0.67, 0,74	0.58	0.49, 0.67	<0.0001

Model 1= Base model

Model 2= Base model, age and race

Model 3= Base model, age, race, systolic blood pressure, ankle-brachial index, LDL, HDL, C-reactive protein, diabetes mellitus, history of coronary artery disease and beta-blocker

Model 4= Base model

Model 5= Base model, age and race

Model 6= Base model, age, race, systolic blood pressure, brachial FMD, LDL, HDL, C-reactive protein, diabetes mellitus, history of coronary artery disease and beta-blocker

TABLE 4

Characteristics of Claudicants by Rutherford Classification.

Characteristics	Rutherford 1 (n=9)	Rutherford 2 (n=12)	Rutherford 3 (n=52)	P-value
Age, Mean (SD), y	63 ± 5	69 ± 10	67 ± 8	0.24
Male Sex	9 (100)	12 (100)	52 (100)	-
Caucasian	5 (56)	10 (83)	38 (73)	0.37
BMI	30 ± 6	27 ± 5	28 ± 5	0.41
Waist-hip ratio (%)	0.99 ± 0.04	1.01 ± 0.05	1.02 ± 0.07	0.38
Systolic Blood Pressure (mm Hg)	129 ± 13	141 ± 24	136 ± 18	0.31
Diastolic Blood Pressure (mmHg)	80 ± 11	77 ± 9	73 ± 9	0.076
Index ABI	0.74 ± 0.11	0.73 ± 0.17	0.67 ± 0.15	0.21
Brachial FMD (%)	12 ± 3	9 ± 3	6 ± 4	0.0001
Comorbidities				
Hypertension	7 (78)	12 (100)	48 (92)	0.18
Hyperlipidemia	9 (100)	11 (92)	46 (88)	0.55
Hx of CAD	1 (11)	4 (33)	21 (40)	0.24
Diabetes Mellitus	5 (56)	4 (33)	22 (42)	0.59
Medications				
Aspirin	6 (67)	9 (75)	32 (62)	0.67
Ace-inhibitor	2 (22)	5 (42)	27 (52)	0.24
B-Blocker	2 (22)	5 (42)	37 (71)	0.008
Statin	8 (89)	12 (100)	45 (87)	0.40
Insulin	1 (11)	3 (25)	9 (17)	0.70
PAD Risk Factors				
History of smoking	8 (89)	12 (100)	49 (94)	0.53
Total Cholesterol (mg/dl)	165 ± 51	166 ± 47	150 ± 39	0.34
LDL (mg/dl)	95 ± 45	89 ± 39	78 ± 34	0.34
HDL (mg/dL)	46 ± 12	49 ± 17	40 ± 12	0.08
Triglycerides (mg/dL)	120 ± 77	140 ± 74	163 ± 105	0.42
Serum creatinine (mg/dL)	0.97 ± 0.17	0.96 ± 0.28	1.09 ± 0.33	0.04
Homocysteine	11.7 ± 3.3	13.3 ± 3.9	14.3 ± 5.4	0.35
CRP (mg/L)	4.5 ± 3.1	4.6 ± 5.5	7.8 ± 23.6	0.82
eGFR	77 ± 12	87 ± 25	76 ± 24	0.38

Values as “mean +/– SD” or “n (%)”

TABLE 5

Adjusted means of brachial artery FMD and adjusted ABI by Rutherford classification in the claudicants.

Brachial Artery FMD

Model	Adjusted Mean Rutherford 1	95% CI	Adjusted Mean Rutherford 2	95% CI	Adjusted Mean Rutherford 3	95% CI	p-value
Model 1	11.7%	9.5, 13.9	8.9%	7.0, 10.8	6.3%	5.3, 7.2	0.0001
Model 2	11.6%	9.4, 13.8	8.8%	6.9, 10.7	6.3%	5.4, 7.2	0.0001
Model 3	11.4%	9.2, 13.6	9.3%	7.4, 11.1	6.6%	5.6, 7.5	0.0008

Ankle-Brachial Index

Model	Adjusted Mean Rutherford 1	95% CI	Adjusted Mean Rutherford 2	95% CI	Adjusted Mean Rutherford 3	95% CI	p-value
Model 4	0.74	0.65, 0.84	0.73	0.64, 0.84	0.67	0.63, 0.71	0.21
Model 5	0.75	0.64, 0.85	0.73	0.65, 0.82	0.67	0.62, 0.71	0.18
Model 6	0.73	0.62, 0.85	0.73	0.64, 0.82	0.68	0.63, 0.73	0.57

Model 1= Base model
Model 2= Base model, age and race
Model 3= Base model, age, race, systolic blood pressure, ankle-brachial index, LDL, HDL, C-reactive protein, diabetes mellitus, history of coronary artery disease and beta-blocker

Model 4= Base model
Model 5= Base model, age and race
Model 6= Base model, age, race, systolic blood pressure, brachial FMD, LDL, HDL, C-reactive protein, diabetes mellitus, history of coronary artery disease and beta-blocker