From the Western Vascular Society

Walking disability in patients with peripheral artery disease is associated with arterial endothelial function

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Objective: **Patients with peripheral artery disease (PAD) have varying degrees of walking disability that do 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 100 PAD 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 the clinic visit and recorded by the investigator-vascular surgeons. Demographic, biochemical, and physiologic parameters were entered into regression equations to determine association with symptom severity.**

Results: **Patients were a mean age of 66 ± 8 years, and 43% had diabetes. Mean FMD was 7.4%, indicating impaired EF. EF progressively declined as Rutherford category increased ($P = .01$). Brachial artery FMD, ABI, systolic blood pressure, C-reactive protein, low-density lipoprotein, high-density lipoprotein, β-blocker use, and a history of diabetes or coronary artery disease were all associated with Rutherford category (all $P < .05$). Multivariable regression showed EF ($P < .02$) and ABI ($P < .0001$) were independently associated with walking disability. When the cohort was restricted to claudicant patients (n = 73), EF remained associated with walking disability after adjustment for other covariates ($P = .0001$).**

Conclusions: **Symptom severity in PAD is multifactorial, reflecting impaired hemodynamics and vascular dysfunction. This is the first report demonstrating that walking disability in PAD is associated with arterial EF. The mechanistic link underlying these observations remains to be defined. (J Vasc Surg 2014;59:1025-34.)**

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Close to one-third of primary care patients aged >70 years 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, nonhealing wounds, and limb loss that confer significant morbidity and mortality. The advanced age and disability of patients with PAD also make them a highly vulnerable population for 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 toward strategies aiming to target the specific pathophysiologic 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.[18,19] 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 (EF), and PAD-related functional

impairment. The goal of this study was to characterize these relationships in a prospective cohort of patients with PAD.

METHODS

The investigator-initiated protocol of this study was approved by the University of California, San Francisco Committee on Human Research. All patients gave informed consent.

Study population and protocol

This cross-sectional study investigated the relationship between EF and walking disability in PAD patients. 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 ABI of <0.9, toe pressures of <70 mm Hg, or imaging confirming >50% stenosis in the lower extremity arteries. Patients without PAD, coronary artery disease (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 ≤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 high-sensitivity C-reactive protein (hsCRP), lipid panel (low-density lipoprotein [LDL], triglycerides, high-density lipoprotein [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, sex, 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 the clinic visit and recorded by the investigator-vascular surgeons (Table I).

Vascular reactivity of brachial arteries. FMD measurement was performed according to current guidelines and standards.[21,22] Subjects were asked to fast (≥8 hours) and abstain from nicotine (≥4 hours) before the examination. The examination took an average of 40 minutes and was performed by the research assistant under the direct supervision of a vascular surgeon. A history of recent medications was recorded.

Table I. 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	

Participants were allowed to rest for 10 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 imaging (HD11; Philips Medical Systems, Bothell, Wash) using a broadband linear array transducer with a 3- to 12-MHz range (L12-3; Philips Medical Systems) until a straight segment with a visible registration structure could be located. The probe was oriented so that the artery was 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.

Before cuff inflation, the baseline diameter of the vessel was recorded for 60 seconds using electrocardiogram-gated image capture software (Brachial Imager; Medical Imaging Applications LLC, Coralville, Iowa). 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 5 minutes. Recording of the B-mode images began 10 seconds before cuff release. Blood-flow velocity was assessed for 30 seconds after cuff release using the methods described above. B-mode images were recorded until 3 minutes after cuff release.

Analyses of the images were performed using continuous edge-detection software (Brachial Analyzer; Medical Imaging Applications LLC). Baseline diameter was recorded as the mean of 60 seconds of data. From hyperemia recordings, the exact moment of cuff release was noted. Hyperemia diameter was calculated using a predetermined time window (55-65 seconds after cuff release). The FMD percentage was calculated as (60-second hyperemia diameter − average baseline diameter/average baseline diameter) × 100.

Time averaged velocity measurements were 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 heartbeats after cuff release. 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 second person and graded on a 6-point scale that included registration structure (landmark), horizontally directed artery, correct longitudinal alignment, clearly visualized near-wall and far-wall intimal medial thickness, 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%.

ABI. ABIs 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 was 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, estimated glomerular filtration rate, albumin, hemoglobin A_{1c} if diabetic, and total cholesterol, triglycerides, LDL, and HDL. Plasma was assayed for these analytes the same day as collection by the SFVAMC laboratory per standard methodology on a Beckman Coulter Analyzer (Beckman Coulter, Miami, Fla). Serum was isolated at the same time points for homocysteine and assayed the same day as collection by the SFVAMC laboratory per standard methodology on an Architect i1000 Analyzer (Abbott Diagnostics, Lake Forest, Ill). The inflammatory marker hsCRP was measured from plasma assayed the same day according to standard methodology (Beckman Coulter Analyzer). 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; claudicant patients, Rutherford 1 to 3; and CLI, Rutherford 4 to 6). Differences in baseline characteristics were compared with the use of analysis of variance for continuous variables and the χ^2 test for dichotomous variables. Because hsCRP had a skewed distribution, it was log-transformed for statistical analyses. For the overall regression models, patients were divided by PAD category (no PAD, Rutherford 0; claudicant patients, Rutherford 1 to 3; and CLI, Rutherford 4 to 6). For regression models in claudicant patients, the Rutherford category was used as a categoric 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 $P < .05$ 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, Tex).

RESULTS

A total of 100 patients participated in this study, including 73 with claudication, 19 with CLI, and eight without PAD. Table II demonstrates the demographics, comorbidities, medications, and PAD risk factors associated with all participants as well as with each of these groups. Patients were a mean age of 66 ± 8 years, 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 II and Figs 1 and 2).

In assessing factors predicting the Rutherford category symptomatology of patients using a univariate analysis, the factors with strongest association included FMD ($P < .0001$), ABI ($P < .0001$), β-blocker use ($P < .0001$), LDL ($P = .001$), HDL ($P = .006$), CRP ($P = .007$), a history of CAD ($P = .002$) or diabetes mellitus ($P = .006$), and systolic blood pressure ($P = .01$). After adjustment for these factors, FMD was still significantly associated with Rutherford category (Table III), with an adjusted mean FMD of 11.4% in controls, 8.0% in claudicant patients, and 5.3% in CLI patients ($P = .02$). Within the same cohort, the ABI also remained significantly associated with Rutherford category after adjustment for factors associated with walking impairment (Table III), whereas none of the other factors remained associated with Rutherford category.

When the cohort was restricted to the 73 claudicant patients, brachial artery FMD decreased with worsening Rutherford category (Table IV). A strong association was also found between FMD and walking impairment ($P = .00011$; Table V). After adjustment for age, race, systolic blood pressure, index ABI, LDL, HDL, CRP, diabetes mellitus, history of CAD, and β-blocker use, FMD remained significantly associated with walking impairment, with an adjusted mean FMD of 11.4% in patients with Rutherford category 1, 9.3% with Rutherford category 2, and 6.6% with Rutherford category 3 symptoms ($P = .0008$; Table V). The ABI did not hold a significant independent association with walking impairment in claudicant patients nor did the other factors (Table V).

FMD and index ABI were not correlated in the entire cohort ($P = .18$), 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. Because the ABI and FMD were not correlated, we

Table II. The baseline characteristics of the population categorized by peripheral arterial disease (*PAD*) category

Characteristics[a]	*All patients (N = 100)*	*No PAD*	*Claudication*	*CLI*	P
		Rutherford 0 (n = 8)	*Rutherford 1-3 (n = 73)*	*Rutherford 4-6 (n = 19)*	
Age, years	66 ± 8	63 ± 8	67 ± 8	67 ± 9	.47
Male sex	100 (100)	8 (100)	73 (100)	19 (100)	…
Caucasian	67 (67)	3 (38)	53 (73)	11 (58)	.09
BMI, kg/m^2	28 ± 5	29 ± 3	28 ± 5	26 ± 6	.47
Waist-to-hip ratio, %	1.0 ± 0.1	0.98 ± 0.07	1.01 ± 0.06	1.0 ± 0.3	.11
Blood pressure, mm Hg					
Systolic	139 ± 22	132 ± 22	136 ± 18	152 ± 29	.008
Diastolic	75 ± 10	77 ± 8	75 ± 9	76 ± 13	.74
Index ABI	0.68 ± 0.2	1.04 ± 0.24	0.69 ± 0.15	0.50 ± 0.15	<.0001
Brachial FMD, %	7 ± 4	11 ± 3	7 ± 4	6 ± 5	.01
Comorbidities					
Hypertension	91 (91)	6 (75)	67 (92)	18 (95)	.24
Hyperlipidemia	87 (87)	6 (75)	66 (90)	15 (79)	.24
History of CAD	39 (39)	1 (13)	26 (36)	12 (63)	.03
Diabetes mellitus	43 (43)	0 (0)	31 (42)	12 (63)	.01
Medications					
Aspirin	66 (66)	3 (38)	47 (64)	16 (84)	.06
ACE inhibitor	42 (42)	1 (13)	34 (47)	7 (37)	.16
β-Blocker	61 (61)	2 (25)	44 (60)	15 (79)	.03
Statin	85 (85)	5 (63)	65 (89)	15 (79)	.10
Insulin	22 (22)	0 (0)	13 (18)	9 (47)	.006
PAD risk factors					
History of smoking	94 (94)	7 (88)	69 (95)	18 (95)	.72
Cholesterol, mg/dL					
Total	153 ± 42	186 ± 41	154 ± 42	134 ± 37	.01
LDL	83 ± 38	116 ± 43	82 ± 36	70 ± 33	.01
HDL	41 ± 13	44 ± 5	43 ± 13	36 ± 13	.09
Triglycerides, mg/dL	151 ± 92	129 ± 53	154 ± 97	149 ± 87	.77
Serum creatinine, mg/dL	1.1 ± 0.3	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.4	.63
Homocysteine, μmol/L	13.3 ± 4.8	10.1 ± 2.5	13.7 ± 4.9	13.2 ± 4.8	.13
CRP, mg/L	10.2 ± 25.7	4.1 ± 2.7	6.8 ± 19.8	25.2 ± 41.1	.02
eGFR, mL/min/1.73 m^2	79 ± 25	83 ± 19	78 ± 23	80 ± 31	.87
Albumin, g/dL	3.9 ± 0.4	4.1 ± 0.1	3.9 ± 0.4	3.6 ± 0.4	.004

ABI, Ankle-brachial index; *ACE,* angiotensin-converting enzyme; *BMI,* body mass index; *CAD,* coronary artery disease; *CLI,* critical limb ischemia; *CRP,* C-reactive protein; *eGFR,* estimated glomerular filtration rate; *FMD,* flow-mediated dilation; *HDL,* high-density lipoprotein; *LDL,* low-density lipoprotein.
[a]Categoric data are shown as number (%) and continuous data as mean ± standard deviation.

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 multifactorial 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, which attracts monocytes to the endothelium, upregulation of tissue factor and proinflammatory cytokines, such as tumor necrosis factor-α, inhibition of nitric oxide, and induction of endothelial adhesion molecules, such as soluble intercellular adhesion molecule 1 and soluble vascular cell adhesion molecule 1, leading to adhesion of monocytes to the endothelium.[19-21]McDermott et al[14-16] previously demonstrated that inflammatory markers including CRP, IL-6, soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule-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.[23,24] Coutinho et al[13] 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 could enhance EF as measured by FMD.[25-27] We therefore confirm through this cross-sectional study that EF assessed by brachial artery FMD can serve as a risk marker for symptom severity and impaired physical activity in patients

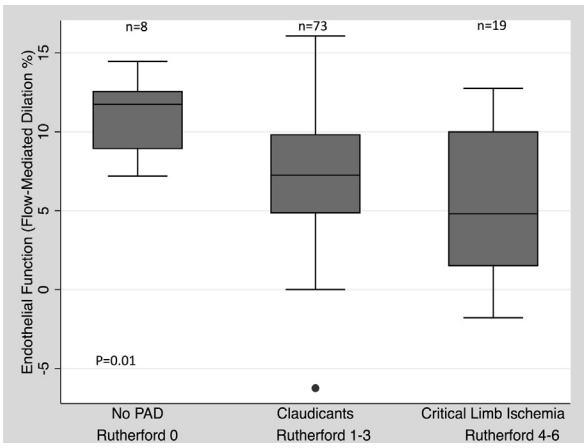

Fig 1. Relationship between symptomatic status of patients and brachial flow-mediated dilation (FMD). Brachial artery FMD is shown by peripheral arterial disease (*PAD*) category in the entire cohort, unadjusted data. *P* value indicates difference between groups using analysis of variance. The *horizontal line* in the middle of each box indicates the median; the *top and bottom borders* of the box mark the 75th and 25th percentiles, respectively, the *top and bottom whiskers* mark the 90th and 10th percentiles, respectively; and the *black circle* indicates outliers.

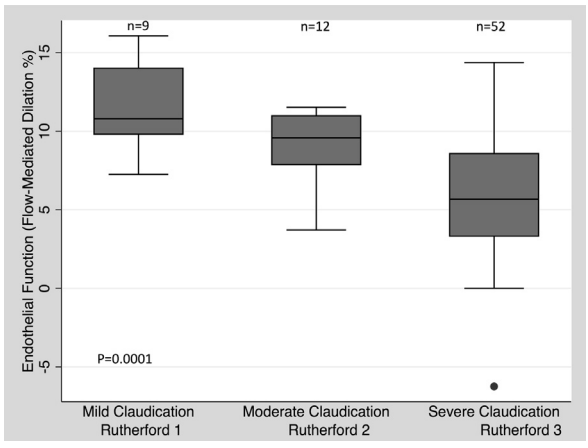

Fig 2. Relationship between symptomatic status of claudicant patients and brachial flow-mediated dilation (FMD) is shown by Rutherford category in patients with claudication, unadjusted data. The *P* value indicates the difference between groups using analysis of variance. The *horizontal line* in the middle of each box indicates the median; the *top and bottom borders* of the box mark the 75th and 25th percentiles, respectively, the *top and bottom whiskers* mark the 90th and 10th percentiles, respectively; and the *black circle* indicates outliers.

with PAD. There is still controversial evidence over the effect of the nitric oxide-mediated vasodilation mechanism and whether nitric oxide supplementation can improve claudication distance and exercise tolerance in PAD patients.[28,29] Thus, further studies are warranted to delve into the physiologic relationship between EF and walking disability as well as therapeutic strategies.

Arterial function, the ABI, and EF. 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 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 epidemiologic 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, a study of PAD patients found a low ABI was 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 they were not directly correlated. This suggests that EF and ABI are both factors that could independently influence symptomatology of patients with PAD.

Although 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 false-negative ABI reading. Exercise testing before and after 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 with rest. If the exercise ABI readings are within normal reference ranges, then leg pain is likely not associated with PAD and can suggest a neurogenic cause or muscle pathology. If the postexercise ABI measurements are in the abnormal range, then PAD is more likely.

β-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. β-Blockade medications, which are among the most common medical therapy prescribed to patients with PAD, serve mainly to mitigate the detrimental effects of uncontrolled hypertension. To this point, 61% of patients

Table III. Adjusted means of brachial artery flow-mediated dilation (*FMD*) and adjusted ankle-brachial index (*ABI*) by peripheral arterial disease (PAD) category in the entire cohort

	Controls (Rutherford 0)		*Claudication (Rutherford 1-3)*		*CLI (Rutherford 4-6)*		
Model	*Adjusted mean, %*	*95% CI*	*Adjusted mean, %*	*95% CI*	*Adjusted mean, %*	*95% CI*	P
Brachial artery FMD							
Model 1	11.0	8.3-13.7	7.4	6.5-8.3	5.8	4.0-7.7	.01
Model 2	10.6	7.8-13.4	7.4	6.5-8.4	5.8	4.0-7.6	.02
Model 3	11.4	8.2-14.6	8.0	7.0-8.9	5.3	3.2-7.5	.02
ABI							
Model 4	1.04	0.93-1.15	0.69	0.65-0.72	0.50	0.42-0.57	<.0001
Model 5	1.05	0.93-1.16	0.68	0.65-0.72	0.50	0.43-0.58	<.0001
Model 6	0.99	0.87-1.11	0.69	0.67-0,74	0.58	0.49-0.67	<.0001

CAD, Coronary artery disease; *CI,* confidence interval; *HDL,* high-density lipoprotein; *LDL,* low-density lipoprotein; *Model 1,* base model; *Model 2,* base model, age and race; *Model 3,* base model, age, race, systolic blood pressure, ABI, LDL, HDL, C-reactive protein, diabetes mellitus, history of CAD and β-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 CAD, and β-blocker.

Table IV. Characteristics of claudication patients by Rutherford classification

Characteristics[a]	*Rutherford 1 (n = 9)*	*Rutherford 2 (n = 12)*	*Rutherford 3 (n = 52)*	P
Age, years	63 ± 5	69 ± 10	67 ± 8	.24
Male sex	9 (100)	12 (100)	52 (100)	…
Caucasian	5 (56)	10 (83)	38 (73)	.37
BMI, kg/m^2	30 ± 6	27 ± 5	28 ± 5	.41
Waist-to-hip ratio, %	0.99 ± 0.04	1.01 ± 0.05	1.02 ± 0.07	.38
Blood pressure, mm Hg				
Systolic	129 ± 13	141 ± 24	136 ± 18	.31
Diastolic	80 ± 11	77 ± 9	73 ± 9	.076
Index ABI	0.74 ± 0.11	0.73 ± 0.17	0.67 ± 0.15	.21
Brachial FMD, %	12 ± 3	9 ± 3	6 ± 4	.0001
Comorbidities				
Hypertension	7 (78)	12 (100)	48 (92)	.18
Hyperlipidemia	9 (100)	11 (92)	46 (88)	.55
History of CAD	1 (11)	4 (33)	21 (40)	.24
Diabetes mellitus	5 (56)	4 (33)	22 (42)	.59
Medications				
Aspirin	6 (67)	9 (75)	32 (62)	.67
ACE inhibitor	2 (22)	5 (42)	27 (52)	.24
β-Blocker	2 (22)	5 (42)	37 (71)	.008
Statin	8 (89)	12 (100)	45 (87)	.40
Insulin	1 (11)	3 (25)	9 (17)	.70
PAD risk factors				
History of smoking	8 (89)	12 (100)	49 (94)	.53
Cholesterol, mg/dL				
Total	165 ± 51	166 ± 47	150 ± 39	.34
LDL	95 ± 45	89 ± 39	78 ± 34	.34
HDL	46 ± 12	49 ± 17	40 ± 12	.08
Triglycerides, mg/dL	120 ± 77	140 ± 74	163 ± 105	.42
Serum creatinine, mg/dL	0.97 ± 0.17	0.96 ± 0.28	1.09 ± 0.33	.04
Homocysteine, μmol/L	11.7 ± 3.3	13.3 ± 3.9	14.3 ± 5.4	.35
CRP, mg/L	4.5 ± 3.1	4.6 ± 5.5	7.8 ± 23.6	.82
eGFR, mL/min/1.73 m^2	77 ± 12	87 ± 25	76 ± 24	.38

ABI, Ankle-brachial index; *ACE,* angiotensin-converting enzyme; *BMI,* body mass index; *CAD,* coronary artery disease; *CRP,* C-reactive protein; *eGFR,* estimated glomerular filtration rate; *FMD,* flow-mediated dilation; *HDL,* high-density lipoprotein; *LDL,* low-density lipoprotein; *PAD,* peripheral arterial disease.

[a]Categoric data are shown as number (%) and continuous data as mean ± standard deviation.

in our cohort were taking a β-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 β-blockers, this includes additional antihypertensive

Table V. Adjusted means of brachial artery flow-mediated dilation (*FMD*) and adjusted ankle-brachial index (*ABI*) by Rutherford classification in the claudicant patients

	Rutherford 1		*Rutherford 2*		*Rutherford 3*		
Model	*Adjusted mean, %*	*95% CI*	*Adjusted mean, %*	*95% CI*	*Adjusted mean, %*	*95% CI*	P
Brachial artery FMD							
Model 1	11.7	9.5-13.9	8.9	7.0-10.8	6.3	5.3-7.2	.0001
Model 2	11.6	9.4-13.8	8.8	6.9-10.7	6.3	5.4-7.2	.0001
Model 3	11.4	9.2-13.6	9.3	7.4-11.1	6.6	5.6-7.5	.0008
ABI							
Model 4	0.74	0.65-0.84	0.73	0.64-0.84	0.67	0.63-0.71	.21
Model 5	0.75	0.64-0.85	0.73	0.65-0.82	0.67	0.62-0.71	.18
Model 6	0.73	0.62-0.85	0.73	0.64-0.82	0.68	0.63-0.73	.57

CAD, Coronary artery disease; *CI*, confidence interval; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *Model 1*, base model; *Model 2*, base model, age and race; *Model 3*, base model, age, race, systolic blood pressure, ABI, LDL, HDL, C-reactive protein, diabetes mellitus, history of CAD, and β-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 CAD, and β-blocker.

medications, statins, and antiplatelet agents), many patients do not experience clinical improvement. In fact, frequency of β-blocker use in our cohort 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, although helpful at reducing cardiovascular risk, are not enough and that there is a need to identify additional therapies that augment the endothelial dysfunction associated with PAD.

Interventions to improve EF. One such promising therapy is ramipril, an angiotensin-converting enzyme inhibitor that was recently shown to improve exercise capacity and enhance quality of life in patients with symptomatic PAD. A 24-week trial, documented an average 77% improvement in pain-free walking and a 123% gain in maximal walking time, corresponding to 75-second and 255-second increases, respectively.[42] The authors proposed that angiotensin-converting enzyme inhibition with ramipril may induce vasodilation by way of reduction in angiotensin II and also improve peripheral blood flow and EF due to bradykinin preservation, thus leading to better functioning. These findings are especially exciting when comparing with cilostazol and pentoxifylline, the only medications currently approved by the United States 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 (PUFAs). In one notable study of young, healthy smokers, Siasos et al[44] found that FMD values significantly improved after oral treatment with 2 g/d of n-3 PUFAs at various time intervals spanning several months. The reasoning underlying this correlation is that fatty acids may improve EF by decreasing the elevated oxidative stress caused by smoking. Supplementation with n-3 PUFAs could lead to recovery of endothelial synthesis of nitric oxide and prostaglandin I_2, as well as vascular smooth muscle cell sensitivity to nitric oxide. These mechanisms are especially relevant to the cohort evaluated in our study, 94% (n = 94) of whom were current or past smokers and had proinflammatory profiles as a result.

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 nitric oxide-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 et al[47] found that 6 months of treadmill exercise led to an increase in FMD, implying improvement in EF and symptom improvement: exercising patients' 6-minute walk distance increased by 20.9 m compared with a decline of 15 m in nonexercising controls. Postexercise ABI was not reported, although 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 arm or leg exercises, compared with 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 because it included only male veterans from SFVAMC referred to a vascular surgery clinic; hence, the findings do not extend to women. It is important to state that this report does not address most patients with PAD, those who are asymptomatic.

The reported Rutherford classification was based on self-report by patients and not verified by a walking impairment questionnaire, which is another limitation. Furthermore, there was no direct functional testing using a treadmill or 6-minute walk test. 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 although 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 cigarette smoking was a factor in the present study because there was no difference in the baseline FMD of smokers vs nonsmokers. This is likely related to the very severe atherosclerotic burden and disease severity of our patient population (ie, 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 effect on symptomatology in patients with PAD.

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AUTHOR CONTRIBUTIONS

Conception and design: MG, KC, WG, JH, JB, CO
Analysis and interpretation: MG, KC, HA, EN, WG, JH, JB, CO
Data collection: MG, KC, HA, CO
Writing the article: MG, EN
Critical revision of the article: KC, HA, WG, JH, JB, CO
Final approval of the article: MG, KC, HA, EN, WG, JH, JB, CO
Statistical analysis: MG, KC, JB, CO
Obtained funding: MG, CO
Overall responsibility: MG

REFERENCES

1. Hirsch AT, Criqui MH, Treat-Jacobson D, Regensteiner JG, Creager MA, Olin JW, et al. Peripheral arterial disease detection, awareness, and treatment in primary care. JAMA 2001;286:1317-24.
2. Fowkes FG, Rudan D, Rudan I, Aboyans V, Denenberg JO, McDermott MM, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. Lancet 2013;382:1329-40.
3. Grenon SM, Vittinghoff E, Owens CD, Conte MS, Whooley M, Cohen BE. Peripheral artery disease and risk of cardiovascular events in patients with coronary artery disease: insights from the Heart and Soul Study. Vasc Med 2013;18:176-84.
4. Ryan AS, Katzel LI, Gardner AW. Determinants of peak V(02) in peripheral arterial occlusive disease patients. J Gerontol A Biol Sci Med Sci 2000;55:B302-6.
5. Brass EP, Hiatt WR. Acquired skeletal muscle metabolic myopathy in atherosclerotic peripheral arterial disease. Vasc Med 2000;5:55-9.
6. Anderson JD, Epstein FH, Meyer CH, Hagspiel KD, Wang H, Berr SS, et al. Multifactorial determinants of functional capacity in peripheral arterial disease: uncoupling of calf muscle perfusion and metabolism. J Am Coll Cardiol 2009;54:628-35.
7. Bauer TA, Brass EP, Hiatt WR. Impaired muscle oxygen use at onset of exercise in peripheral arterial disease. J Vasc Surg 2004;40:488-93.
8. Charters Y, Grimble RF. Effect of recombinant human tumour necrosis factor alpha on protein synthesis in liver, skeletal muscle and skin of rats. Biochem J 1989;258:493-7.
9. Goodman MN. Tumor necrosis factor induces skeletal muscle protein breakdown in rats. Am J Physiol 1991;260:E727-30.
10. Goodman MN. Interleukin-6 induces skeletal muscle protein breakdown in rats. Proc Soc Exp Biol Med 1994;205:182-5.
11. Brewer LC, Chai HS, Bailey KR, Kullo IJ. Measures of arterial stiffness and wave reflection are associated with walking distance in patients with peripheral arterial disease. Atherosclerosis 2007;191:384-90.
12. Payvandi L, Dyer A, McPherson D, Ades P, Stein J, Liu K, et al. Physical activity during daily life and brachial artery flow-mediated dilation in peripheral arterial disease. Vasc Med 2009;14:193-201.
13. Coutinho T, Rooke TW, Kullo IJ. Arterial dysfunction and functional performance in patients with peripheral artery disease: a review. Vasc Med 2011;16:203-11.
14. McDermott MM, Lloyd-Jones DM. The role of biomarkers and genetics in peripheral arterial disease. J Am Coll Cardiol 2009;54:1228-37.
15. McDermott MM, Liu K, Ferrucci L, Tian L, Guralnik JM, Green D, et al. Circulating blood markers and functional impairment in peripheral arterial disease. J Am Geriatr Soc 2008;56:1504-10.
16. McDermott MM, Ferrucci L, Liu K, Criqui MH, Greenland P, Green D, et al. D-dimer and inflammatory markers as predictors of functional decline in men and women with and without peripheral arterial disease. J Am Geriatr Soc 2005;53:1688-96.
17. McDermott MM, Liu K, Guralnik JM, Ferrucci L, Green D, Greenland P, et al. Functional decline in patients with and without peripheral arterial disease: predictive value of annual changes in levels of C-reactive protein and D-dimer. J Gerontol A Biol Sci Med Sci 2006;61:374-9.
18. Szuba A, Oka RK, Harada R, Cooke JP. Limb hemodynamics are not predictive of functional capacity in patients with PAD. Vasc Med 2006;11:155-63.
19. Parr B, Noakes TD, Derman EW. Factors predicting walking intolerance in patients with peripheral arterial disease and intermittent claudication. S Afr Med J 2008;98:958-62.
20. Grenon SM, Gagnon J, Hsiang Y. Video in clinical medicine. Ankle-brachial index for assessment of peripheral arterial disease. N Engl J Med 2009;361:e40.
21. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257-65.
22. Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol 2011;300:H2-12.
23. Joras M, Poredos P. The association of acute exercise-induced ischaemia with systemic vasodilator function in patients with peripheral arterial disease. Vasc Med 2008;13:255-62.

24. Brevetti G, Schiano V, Chiariello M. Endothelial dysfunction: a key to the pathophysiology and natural history of peripheral arterial disease? Atherosclerosis 2008;197:1-11.
25. Kwon HR, Min KW, Ahn HJ, Seok HG, Lee JH, Park GS, et al. Effects of aerobic exercise vs. resistance training on endothelial function in women with type 2 diabetes mellitus. Diabetes Metab J 2011;35:364-73.
26. Ades PA, Savage PD, Lischke S, Toth MJ, Harvey-Berino J, Bunn JY, et al. The effect of weight loss and exercise training on flow-mediated dilatation in coronary heart disease: a randomized trial. Chest 2011;140:1420-7.
27. Mika P, Konik A, Januszek R, Petriczek T, Mika A, Nowobilski R, et al. Comparison of two treadmill training programs on walking ability and endothelial function in intermittent claudication. Int J Cardiol 2013;168:838-42.
28. Gresele P, Migliacci R, Arosio E, Bonizzoni E, Minuz P, Violi F, et al. Effect on walking distance and atherosclerosis progression of a nitric oxide-donating agent in intermittent claudication. J Vasc Surg 2012;56:1622-8. 28 e1-5.
29. Kenjale AA, Ham KL, Stabler T, Robbins JL, Johnson JL, Vanbruggen M, et al. Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. J Appl Physiol 2011;110:1582-91.
30. Parmenter BJ, Raymond J, Fiatarone Singh MA. The effect of exercise on haemodynamics in intermittent claudication: a systematic review of randomized controlled trials. Sports Med 2010;40:433-47.
31. Vogt MT, McKenna M, Anderson SJ, Wolfson SK, Kuller LH. The relationship between ankle-arm index and mortality in older men and women. J Am Geriatr Soc 1993;41:523-30.
32. Newman AB, Shemanski L, Manolio TA, Cushman M, Mittelmark M, Polak JF, et al. Ankle-arm index as a predictor of cardiovascular disease and mortality in the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol 1999;19:538-45.
33. McKenna M, Wolfson S, Kuller L. The ratio of ankle and arm arterial pressure as an independent predictor of mortality. Atherosclerosis 1991;87:119-28.
34. Diehm C, Lange S, Darius H, Pittrow D, von Stritzky B, Tepohl G, et al. Association of low ankle brachial index with high mortality in primary care. Eur Heart J 2006;27:1743-9.
35. Brevetti G, Silvestro A, Di Giacomo S, Bucur R, Di Donato A, Schiano V, et al. Endothelial dysfunction in peripheral arterial disease is related to increase in plasma markers of inflammation and severity of peripheral circulatory impairment but not to classic risk factors and atherosclerotic burden. J Vasc Surg 2003;38:374-9.
36. Silvestro A, Scopacasa F, Ruocco A, Oliva G, Schiano V, Zincarelli C, et al. Inflammatory status and endothelial function in asymptomatic and symptomatic peripheral arterial disease. Vasc Med (London, England) 2003;8:225-32.
37. Lind L. Arterial stiffness but not endothelium-dependent vasodilation, is related to a low ankle-brachial index in the elderly—The Prospective Investigation of the Vasculature in the Uppsala Seniors (PIVUS) Study. Open Atheroscler Thromb J 2008;I:1-5.
38. McDermott MM, Liu K, Greenland P, Guralnik JM, Criqui MH, Chan C, et al. Functional decline in peripheral arterial disease: associations with the ankle brachial index and leg symptoms. JAMA 2004;292:453-61.
39. McDermott MM, Greenland P, Liu K, Guralnik JM, Celic L, Criqui MH, et al. The ankle brachial index is associated with leg function and physical activity: the Walking and Leg Circulation Study. Ann Intern Med 2002;136:873-83.
40. McPhail IR, Spittell PC, Weston SA, Bailey KR. Intermittent claudication: an objective office-based assessment. J Am Coll Cardiol 2001;37:1381-5.
41. Garber CE, Monteiro R, Patterson RB, Braun CM, Lamont LS. A comparison of treadmill and arm-leg ergometry exercise testing for assessing exercise capacity in patients with peripheral arterial disease. J Cardiopulm Rehabil 2006;26:297-303.
42. Ahimastos AA, Walker PJ, Askew C, Leicht A, Pappas E, Blombery P, et al. Effect of ramipril on walking times and quality of life among patients with peripheral artery disease and intermittent claudication: a randomized controlled trial. JAMA 2013;309:453-60.
43. Stevens JW, Simpson E, Harnan S, Squires H, Meng Y, Thomas S, et al. Systematic review of the efficacy of cilostazol, naftidrofuryl oxalate and pentoxifylline for the treatment of intermittent claudication. Br J Surg 2012;99:1630-8.
44. Siasos G, Tousoulis D, Oikonomou E, Zaromitidou M, Verveniotis A, Plastiras A, et al. Effects of omega-3 fatty acids on endothelial function, arterial wall properties, inflammatory and fibrinolytic status in smokers: a cross over study. Int J Cardiol 2013;166:340-6.
45. Hamburg NM, Balady GJ. Exercise rehabilitation in peripheral artery disease: functional impact and mechanisms of benefits. Circulation 2011;123:87-97.
46. Izquierdo-Porrera AM, Gardner AW, Powell CC, Katzel LI. Effects of exercise rehabilitation on cardiovascular risk factors in older patients with peripheral arterial occlusive disease. J Vasc Surg 2000;31:670-7.
47. McDermott MM, Ades P, Guralnik JM, Dyer A, Ferrucci L, Liu K, et al. Treadmill exercise and resistance training in patients with peripheral arterial disease with and without intermittent claudication: a randomized controlled trial. JAMA 2009;301:165-74.
48. Zwierska I, Walker RD, Choksy SA, Male JS, Pockley AG, Saxton JM. Upper- vs lower-limb aerobic exercise rehabilitation in patients with symptomatic peripheral arterial disease: a randomized controlled trial. J Vasc Surg 2005;42:1122-30.
49. Munzel T, Sinning C, Post F, Warnholtz A, Schulz E. Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. Ann Med 2008;40:180-96.
50. Neunteufl T, Heher S, Kostner K, Mitulovic G, Lehr S, Khoschsorur G, et al. Contribution of nicotine to acute endothelial dysfunction in long-term smokers. J Am Coll Cardiol 2002;39:251-6.

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DISCUSSION

Dr Larry Kraiss (*Salt Lake City, Utah*). Congratulations to Ms Chong who very ably presented this paper. I also thank the authors for a timely submission of their paper for my review.

The UCSF group is to be commended for reminding us that PAD is in reality a systemic disease. They report that self-reported walking impairment correlates best with objectively measured endothelial function, which is a systemic condition as opposed to the ABI, which might be considered a limb-specific condition. Walking impairment is an endpoint that is sensitive to many factors, only one of which is the ABI or the degree of hemodynamic impairment that the limb experiences. Also, I think we have all had patients who underwent intervention for claudication with an improvement in ABI but a disappointing response in walking distance. So, this particular conclusion, on its face, is not too difficult to accept, but I do have some reservations about the data supporting this conclusion.

First, although the study population is extensively phenotyped in terms of endothelial function, ABI, and multiple biochemical parameters, the clinical characterization is suboptimal. As we have heard before during this meeting, the venerable Rutherford classification has probably outlived its usefulness, so I question the accuracy of self-reported walking distance in terms of "blocks." There is no information about whether these self-reported outcomes are accurate or reproducible. Your study would be greatly strengthened by an objective measure of walking impairment, such as the 6-minute walk test. While the authors acknowledge this limitation, it remains a major drawback to the study's impact.

Ms Karen Chong. We acknowledge this as a limitation of our study. We are therefore excited to launch our trial that will have a 6-minute walk test as an outcome in addition to self-reported Rutherford and the WIQ.

Dr Kraiss. Second, the authors report that endothelial function as measured by brachial artery FMD is the only parameter that correlates with severity of claudication classified by Rutherford category. However, in the manuscript, there is a 100% overlap between endothelial function scores for group II compared to group III. This challenges my statistical "smell" test.

Finally, the authors report that endothelial function did not correlate with ABI but the *P* value for this relationship is .21. So, there is an 80% chance that ABI and endothelial function really do correlate. Since there are so few subjects in the Rutherford class I and II categories, I wonder if it is really true that ABI and endothelial function do not correlate or whether this is in essence a type II error.

These are issues that I will let the authors address in the JVS editorial process, as I am not sure that a discussion around these points will be all that informative this morning. I do have four straightforward questions for the authors:

1. How were patients selected for the study? Were they consecutive? Were they new or established patients, or both?
2. Did inflow or outflow location of disease correlate with endothelial function?
3. Had any patients in the study received previous treatment directed at claudication?
4. Have any of the study patients subsequently received treatment for claudication? If so, did endothelial function predict treatment outcome?

This is a worthwhile area of study and I encourage the authors to continue their investigations. I suspect that they are on to something that may ultimately help us better select claudicants who will benefit the most from intervention.

Ms Chong. To answer your questions:

1. We essentially attempted to recruit any patient that came through our clinic with PAD, provided that they fit our eligibility criteria and provided consent. This included both new and established patients.
2. We have not yet looked at the location of the disease, but that is actually the next direction for us. We plan to look at angiographic or MR imaging to determine location of lesions and quantity of collaterals to see if these factors might correlate with endothelial function or walking disability.
3. There were patients that had prior revascularization, but this was the minority. In those cases, we based our clinical Rutherford category on their symptoms prior to any revascularization to ensure that their native symptoms would be captured.
4. Study patients certainly have subsequently received treatment for claudication. However, we have not collected these data, but we do have access to it. It would be interesting to see if endothelial function does predict treatment outcome. For now, our lab, the Vascular Integrated Physiology and Experimental Therapeutics Lab (VIPERx), is focusing on establishing a modifiable risk factor in patients with claudication.