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MRI Perfusion Index as a Measure of Peripheral Arterial Disease Severity

By

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Master of Science

Clinical Research

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MRI Perfusion Index as a Measure of Peripheral Arterial Disease Severity

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Abstract

MRI Perfusion Index as a Measure of Peripheral Arterial Disease Severity
By Matthew L. Topel

Background: Objective and quantitative evaluation of the functional impairment caused by peripheral arterial disease (PAD) is difficult and frequently assessed through proxy measurements such as the ankle-brachial index (ABI). While ABI is both sensitive and specific for the diagnosis of PAD, its value in assessing disease severity and monitoring disease improvement is poor. Recently, gadolinium-enhanced first-pass magnetic resonance imaging (MRI) of the lower extremities has emerged as a new method to assess perfusion in peripheral muscles immediately following peak exercise.

Objective: To demonstrate that calf muscle perfusion index (PI) measured with MRI is associated with PAD severity, as measured by peak treadmill walking time (PWT).

Methods: 82 subjects with PAD were included in the study. Subjects exercised until fatigue using an MR-compatible plantar-flexion ergometer. Images were acquired immediately following peak exercise using a dynamic, first-pass, dual-contrast sequence. PI was calculated as a ratio of muscle perfusion to arterial flow. Graded treadmill exercise testing was performed using the Gardner protocol. PWT, pre- and post-exercise ABI were recorded for each patient. Demographic and historical information were collected through screening questionnaires.

Results: 64 patients completed both the MRI and treadmill test. A 1 standard deviation increase in PI was associated with a 20.7% increase in PWT (95% CI, 3.42-40.9%; R^2=0.087, *P*=0.018). PI was not correlated with pre- or post-exercise ABI. After adjusting for ABI, PI remained a significant predictor of PWT (F=8.91, *P*=0.004). Stratified analysis of potential interaction showed that the association between PI and PWT was significant only at "High" ABI levels (pre-exercise, R^2=0.263, *P*=0.004; post-exercise R^2=0.211, *P*=0.008) and in individuals without previous lower extremity vascular intervention (R^2=0.105, *P*=0.042).

Conclusions: MR-based calf muscle PI is associated with PAD severity, as measured by PWT. Additionally, the association between PI and PWT appears stronger at "High" ABI levels and in subjects without a history of previous lower extremity vascular intervention. PI may be a valuable tool to objectively and quantitatively assess muscle perfusion in a PAD population with borderline normal ABI and no history of lower extremity vascular intervention.

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INTRODUCTION

Peripheral arterial disease (PAD) is an acquired disease of the lower extremity circulation that is most often secondary to atherosclerosis. It is typified by intermittent claudication, which is the occurrence of reproducible pain in specific groups of leg muscles that is relieved by rest; however, only a fraction of individuals with PAD exhibit intermittent claudication, and reliance on clinical symptoms alone results in widespread under-diagnosis of PAD in the general population (1, 2). The presence of PAD is an independent predictor of all-cause mortality and is associated with an increased incidence of other cardiovascular events such as stroke and myocardial infarction; furthermore, increasing severity of PAD is associated with worse outcomes (3-6).

The ankle-brachial index (ABI) is a well-validated clinical tool used to diagnose PAD and to grade its severity; however, there is some indication that it may be biased toward detecting more advanced disease (6-8). Recently, magnetic resonance (MR)-based techniques have been developed to determine blood flow and skeletal muscle perfusion in patients with PAD (9-11). An MR-derived perfusion index (PI) may be a more sensitive assessment of disease severity in patients with PAD, may be a more objective measure for tracking progress following an intervention for PAD, and could ultimately provide both an anatomical and functional evaluation of disease burden (12).

The relationship between PI, ABI and the symptomatic severity of PAD has not been explored before. In subjects with PAD enrolled in a phase II clinical trial, we performed a cross-sectional analysis between PI, ABI and PAD severity, measured as peak treadmill walking time (PWT) to determine if PI is associated with PWT. Additionally, we sought to determine whether PI was associated with PWT, controlling

for ABI, and if the association between PI and PWT depends upon the level of ABI and/or a history of lower extremity vascular intervention. We hypothesize that PI is associated with PWT, even when controlling for ABI.

BACKGROUND

Peripheral arterial disease (PAD) affects approximately 10 million Americans, or 5% of the US population over 40 years of age, and upwards of 27 million individuals in Europe and North America combined (3, 13, 14). Pathophysiologically, PAD is an inability of the vascular system to adequately supply the peripheral tissues with oxygenated blood and is often a result of obstructive atherosclerosis affecting the lower limb arteries (15). While the majority of patients are asymptomatic, the typical clinical manifestation is intermittent claudication, which is reproducible leg-muscle discomfort on exertion that is relieved by rest. Intermittent claudication affects less than 20% of individuals with PAD and portends a worse prognosis (1, 2).

Risk factors for the development of PAD are similar to other atherosclerotic cardiovascular diseases and include advanced age, smoking, diabetes, hypertension, and hyperlipidemia. Notably, smoking and diabetes have a stronger impact on the development and severity of PAD (3, 14, 16, 17). There is significant co-prevalence of PAD and other atherosclerotic diseases; up to 80% of individuals with PAD have significant coronary artery disease and up to 25% have significant carotid artery disease (6). The risk of all-cause mortality is directly associated with the severity of PAD (5). Individuals with PAD have a 5-7% annual incidence of major adverse cardiovascular events including stroke, myocardial infarction and death (3, 13, 18, 19).

A number of diagnostic methods are employed in the assessment of PAD (6, 20). A resting ankle-brachial index (ABI) is the primary clinical tool used to establish a diagnosis of PAD. It is calculated as the ratio of blood pressure in the lower extremities to blood pressure in the upper extremities. Normal values for resting ABI range from 1.00

to 1.40, while an ABI ≤ 0.90 indicates PAD. An ABI ≤ 0.90 confers almost 95% sensitivity for angiographically-positive PAD and approaches 100% specificity in ruling out healthy individuals (21). There is an increased risk of mortality and other co-morbid cardiovascular conditions with decreasing ABI (22-24). Further advantages of the ABI measurement include its relative ease of performance in the clinic setting and its cost-effectiveness (6). Despite its widespread use and many benefits, the ABI does have a number of limitations. While ABI is associated with walking velocity and endurance (25), it does not quantify functional impairment and does not define the anatomic severity of atherosclerosis in PAD. The accuracy of ABI is poor in non-compressible arteries, which are typically found in elderly patients and in those with diabetes (26), populations in which PAD is highly prevalent. The ABI may be biased towards detecting more severe, distal disease and may under-diagnose PAD in patients with mild disease. In one study comparing ABI and Duplex ultrasound, a sensitive, non-invasive measure of PAD severity, investigators found 100% agreement between the two measures at ABI < 0.6 and only 76% agreement at ABI ≥ 0.9 (7). Another study showed that 31% of patients with normal resting ABI had an abnormal post-exercise ABI (8). While ABI for the diagnosis of PAD has near-perfect sensitivity and specificity, it becomes much less sensitive and specific when used as a tool for monitoring disease progression in follow-up for patients who have undergone a lower extremity revascularization intervention (27). Finally, in patients with severe, proximal disease, the ABI may be inappropriately high, especially if an extensive collateral circulation is present (21).

Patients with claudication-like pain but with equivocal findings on the resting ABI may undergo exercise testing to establish a diagnosis of PAD. Pre- and post-exercise

ABI are recorded, and a reduction in ABI with exercise indicates presence of PAD. Although various cut-offs for absolute and relative reductions in ABI have been proposed for the diagnosis of PAD, there is currently no consensus (6). Measures of walking performance are independent predictors of mortality in patients with PAD and provide prognostic information beyond ABI alone (28). In addition to providing an alternative method for PAD diagnosis, treadmill exercise testing is a useful method for assessing functional impairment and for objectively monitoring improvement in performance and function during a selected intervention. Although one may expect that exercise testing measures and ABI are associated, this is not always the case (29). Therefore, measures of exercise testing, such as "peak walking time" and "time to claudication," provide valuable additional information in the assessment of PAD severity and improvement. Limitations of exercise testing, with and without ABI measurements, include acquisition and training of staff, equipment purchase and maintenance costs, and the lack of a uniform, standard exercise protocol for exercise testing (6).

Contrast angiography is the "gold standard" for defining vascular disease of the lower extremities and is reserved for patients with severe PAD for whom medical therapy is insufficient to manage symptoms and who are undergoing evaluation for surgery. By comparison to ABI, contrast angiography is invasive and requires exposure to radiation and contrast dye with nephrotoxic properties (6). Magnetic resonance angiography (MRA) is an alternative technique for assessing the anatomical severity of lower extremity vascular lesions. A meta-analysis comparing MRA to angiography in the detection of >50% stenoses revealed that each had a sensitivity and specificity between 90% and 100%; notably, the accuracy of MRA was greatest using gadolinium contrast

enhancement (30). A more recent study reports 91 – 97% agreement between MRA and angiography, and a specific study assessing the utility of MRA in preoperative planning demonstrated 90% or greater agreement between MRA and angiography during preoperative planning (31, 32). MRA is generally limited by the effects of its use of magnets; namely, metal stents may cause artifact and patients with implantable devices, such as pacemakers and defibrillators, cannot undergo imaging. Additionally, MRA tends to overestimate the degree of vessel stenosis, and the use of gadolinium contrast can infrequently cause renal toxicity (33, 34).

In an effort to better quantify blood flow and tissue perfusion to the lower extremities, a variety of new magnetic resonance (MR)-based techniques have been developed and evaluated (9-11). These include the use of a gadolinium-based MR contrast agent to measure perfusion during exercise or post-ischemic reactive hyperemia. The benefit of exercise over reactive hyperemia is that exercise produces greater peak blood flows and more accurately mimics the physiological stress experienced in claudication (35). Additionally, regional flow in a reactive hyperemia model is determined by muscle fiber content and capillary density, whereas the perfusion of muscle tissue following peak exercise is a result of active muscle recruitment (36). Although a novel technique for assessing muscle perfusion in the setting of PAD, first-pass gadolinium-enhanced MR perfusion imaging has been utilized for years in the evaluation, management and follow-up of patients with coronary artery disease and is a well-validated tool for assessing myocardial perfusion (37-39). First-pass gadolinium-enhanced MR perfusion of the lower limbs has been used to calculate a perfusion index (PI), which is the ratio of regional muscle perfusion to arterial flow at peak-exercise. In a

small study comparing subjects with PAD to normal control subjects, PI was able to discriminate between the two populations (9).

In summary, PAD is a increasingly common manifestation of atherosclerosis and results in significant morbidity and mortality. While traditional methods for diagnosing PAD and assessing its severity are well-established, they tend to measure surrogate markers of the ultimate target: lower limb muscle perfusion. Because of this, these traditional measures are prone to error, especially at lower peripheral perfusion states seen in severe PAD. MR PI is an emerging tool to assess the relationship between muscle perfusion and arterial flow in the lower limbs and may represent a better, more sensitive measure of PAD severity and response to therapy. Very little is known about the relationship between PI, ABI and functional or symptomatic severity of PAD. In the present study, we aim to define these relationships by using peak walking time (PWT) as a measure of the functional severity of PAD. We also aim to determine whether the relationship between PI and PAD severity is modified by the level of ABI impairment and/or a history of lower extremity vascular intervention.

METHODS

Hypothesis: Lower limb muscle MR perfusion index (PI) is associated with severity of PAD, measured as peak walking time (PWT).

Study Questions: Is lower limb muscle MR PI associated with severity of PAD? Is PI associated with the severity of PAD after accounting for ABI? Is the association between PI and severity of PAD modified by: a) the level of ABI and/or b) previous lower extremity vascular intervention?

Study Design: A cross-sectional study of baseline data from participants enrolled in the GPAD-2 study, a single-center phase II randomized, double-blind, placebo-controlled trial assessing the benefit of granulocyte-macrophage colony stimulating factor (GM-CSF) for the mobilization of endothelial progenitor cells in PAD (ClinicalTrials.gov Identifier: NCT01041417).

Study Population: A number of inclusion and exclusion criteria were used to enroll subjects into the study (**Appendix A**). Men and women between the ages of 21 and 80 with a previous diagnosis of PAD, clinically stable intermittent claudication, and resting ABI < 0.85 (or a 20% reduction in ABI from pre- to post-exercise) on screening were eligible. Relevant exclusion criteria include recent lower extremity vascular surgery, recent participation in a structured exercise program and non-claudication causes of exercise limitation, such as asthma or osteoarthritis. Subjects were recruited from multiple sites in the Atlanta area, primarily the Atlanta Veterans Affairs Medical Center (VAMC), Emory University Hospital (EUH) and Grady Memorial Hospital (GMH). Within the 1:1 randomization structure of the clinical trial, subjects were further sequentially selected to undergo the MR perfusion protocol. Of the 296 patients

consented for participation in the trial, 159 subjects were enrolled; 82 subjects received the MR perfusion protocol (**Figure 1**).

The Emory University Institutional Review Board approved the study and all participants provided written informed consent. Furthermore, a Data and Safety Monitoring Board reviewed safety data throughout the study.

Measurements: Demographic and historical data were obtained by patient self-report from screening questionnaires, including: age (in years), race, gender, smoking history (in pack-years) and history of diabetes, hypertension, hyperlipidemia, myocardial infarction, stroke and lower extremity vascular intervention.

Subjects further underwent multiple studies to determine the main outcome and predictor variables for analysis. The following variables were calculated:

Perfusion index (Main predictor): Acquisition of the PI using a first-past gadolinium-enhanced MR protocol has been previously described (9). Subjects were placed in the supine position in the MR scanner, and a plantar-flexion exercise ergometer was positioned at the subject's feet for use during the exercise component of the protocol.

Localizer scans were initially acquired with the subject at rest. Next, the patient was instructed to push against the ergometer at a rate of 10-12 sec^{-1} until exhaustion and/or limiting symptoms occurred; this exercise was meant to simulate claudication pain during walking. Immediately following exercise cessation, 0.1 mmol/kg of gadolinium contrast dye was infused intravenously at a rate of 4.0 mL/sec, with simultaneous initiation of 100 dynamic image

acquisitions for determination of the perfusion index. An additional 0.1 mmol/kg gadolinium infusion was performed for acquisition of MRA imaging.

Post-imaging analysis and processing was performed by a single operator using OsiriX, an open-source imaging software tool (available at http://www.osirix-viewer.com/Downloads.html), and MATLAB (MathWorks, Natick, MA). Regions of interest (ROIs) at the level of the popliteal artery and the calf muscle were identified for analysis following infusion of contrast (**Figure 2a**). The ROIs at the level of the popliteal artery (blue circles) allow for measurement of the arterial input function (**Figure 2b**), while the ROIs at the level of the calf muscle (red circles) allow for measurement of the muscle perfusion (**Figure 2c**). Time-intensity curves were created for each ROI to determine the values of muscle perfusion and the arterial input function, and the peak slope of signal intensity was used for each measurement (**Figure 3**). Finally, PI was calculated as the muscle perfusion divided by arterial input function.

A total of 30 randomly selected data sets were re-analyzed for intraobserver variability, with > 98% agreement.

Ankle-brachial index (Secondary predictor): The procedure for calculating ABI has been described previously (6, 20, 40). With the patient supine, bilateral upper and lower extremity blood pressure cuffs were inflated to suprasystolic pressures. Doppler flow signals were used to detect the reappearing perfusion during incremental lowering of the cuff pressure. Systolic blood pressure measurements were taken at the upper (brachial artery) and lower (dorsalis pedis and posterior tibial arteries) extremities. Leg-specific ABI was calculated as a

ratio of the highest lower extremity pressure in each leg divided by the highest upper extremity pressure in either arm (**Figure 4**). ABI was measured at rest and immediately following treadmill exercise testing.

Peak walking time (Outcome): Graded treadmill exercise testing was performed using the Gardner protocol; the treadmill speed was set at 2 mph, while the initial grade was set at 0° and increased by 2° every 2 minutes of walking. The peak walking time (PWT) was the time (in seconds) at which exercise was terminated because of severe claudication.

Sample Size and Power Calculations: No sample size calculation or power analysis was performed explicitly for the current study; however, both were done for the GPAD-2 protocol. Based on preliminary data from a collaborating group at the University of Virginia (9), the estimated standard deviation of the perfusion index was 0.15. Assuming an α=0.05 and power of 80%, 40 subjects in both the placebo and treatment groups (for a total of 80 subjects) were needed to detect a 0.11 or greater change in the perfusion index.

Statistical Analysis: Subject characteristics were summarized as mean and standard deviation (SD) for normal continuous variables, median and interquartile range (IQR) for skewed continuous variables, and number and frequency (%) for categorical variables. The hypothesis was tested using peak walking time (PWT) as the outcome variable and perfusion index (PI) as the predictor variable. Assumptions for linear regression were assessed; in order to satisfy the condition of linearity, PWT was logarithmically transformed as the outcome of interest, and data were reported in the multiplicative model as percent changes in PWT rather than absolute changes in PWT. Univariate associations between PWT, PI and potential predictors were assessed for the primary

hypothesis. These associations were determined using a linear regression model. Partial F-tests were calculated to assess the association between PWT and PI, conditioning on ABI. We pre-determined three interaction variables for analysis: pre-exercise ABI, post-exercise ABI and history of lower extremity vascular intervention. Based on prior evidence that resting ABI has better agreement with perfusion at lower levels (7), we performed a median split on pre-exercise ABI and stratified our subjects into "Low" ($ABI \leq 0.6$) and "High" ($ABI > 0.6$) groups. We also performed a median split on post-exercise ABI for "Low" ($ABI \leq 0.31$) and "High" ($ABI > 0.31$) groups. Because measurement of PI requires identifying certain anatomic landmarks on MRI imaging, presence or absence of previous lower extremity vascular intervention was also assessed for interaction. Scatter plots were generated to visually inspect for possible interaction, and a stratified univariate regression was performed on PWT and PI at each level of the interaction variable. A multivariate model was used to determine the statistical significance of interaction. All analysis was performed using SAS v. 9.3 (Cary, NC).

RESULTS

Patient demographics and characteristics

A total of 82 subjects enrolled in the GPAD-2 study underwent the MR perfusion protocol. The mean age of subjects was 63.9 ± 8.2 years, and there were no significant differences between the groups who did and did not undergo MR perfusion testing. Of the subjects completing the study, 68 had interpretable scans; there were no significant differences between the interpretable and non-interpretable groups. The MR perfusion protocol cohort was demographically similar to previous PAD study populations at this institution (41). Compared to individuals with PAD in the general population, this cohort had a higher prevalence of males (90%), hypertension (96%), and hyperlipidemia (84%) (**Table 1**). The mean perfusion index (PI) of subjects was 0.09 ± 0.05, and the median peak walking time (PWT) of subjects was 257 seconds (150 - 332 seconds).

Predictors of peak walking time and perfusion index

Potential predictors of peak walking time (PWT) were assessed in a univariate linear model (**Table 2**). A one standard deviation (1 S.D.) increase, which represents a 0.05 unit increase, in the perfusion index (PI) was associated with a 20.7% increase in PWT (95% CI, 3.42 - 40.9%; R^2=0.087, *P*=0.018) (**Figure 5**). Ankle-brachial index (ABI) was also associated with PWT; a 0.1 unit increase in pre-exercise ABI was associated with a 19.5% increase in PWT (95% CI, 10.1 - 29.1%; R^2=0.237, *P*<0.001), while a 0.1 unit increase in post-exercise ABI was associated with a 15.4% increase in PWT (95% CI, 8.49 - 22.8%; R^2=0.260, *P*<0.001). Race and smoking were borderline significantly associated with PWT; Caucasian race (vs. any other race) was associated

with a 24.1% decrease in PWT (95% CI, -42.7 - 0.40%; R^2=0.054, *P*=0.054), and a 10 pack-year increase in smoking was associated with a 10.0% decrease in PWT (95% CI, -19.1 - 0.13%; R^2=0.061, *P*=0.053).

Association of perfusion index and peak walking time, given ankle-brachial index

The association between PI and PWT, adjusting for ABI, was assessed using the partial F-test (**Table 3**). Individually, PI explained approximately 9% of the variance in PWT, while pre-exercise ABI explained approximately 24% of the variance in PWT. The multivariate model with both PI and pre-exercise ABI as predictors explained approximately 34% of the variance of PWT (*P*<0.001). The partial F-test statistic for inclusion of PI in the associative model, given pre-exercise ABI, was significant (F=8.91, *P*=0.004). This finding was replicated for post-exercise ABI, which explained approximately 26% of the variance of PWT. The multivariate model with both PI and post-exercise ABI as predictors explained approximately 38% of the variance of PWT (*P*<0.001), and the partial F-test statistic for inclusion of PI in the associative model, given post-exercise ABI, was significant (F=11.76, *P*=0.001).

Assessing interaction between the level of ankle-brachial index and perfusion index

The analysis of covariance scatter plot for PI and PWT, stratified by level of pre-exercise ABI, demonstrated different slopes (**Figure 6**). A stratified univariate analysis was performed for the association of PI with PWT at each level of pre-exercise ABI (**Table 4**). Within the “High” category, a 1 S.D. increase in PI was associated with a 29.3% increase in PWT (95% CI, 10.5 - 51.3%; R^2=0.263, *P*=0.002); however, within the

"Low" category, no association was observed between PI and PWT (10.2%; 95% CI, -15.5 - 43.7%; R^2=0.020, *P*=0.460). Although suggested by the scatter plot and the stratified analysis, the interaction of PI and pre-exercise ABI group was non statistically significant (*P*=0.345).

Post-exercise ABI group was also assessed for interaction with PI. The analysis of covariance scatter plot for PI and PWT, stratified by level of post-exercise ABI, demonstrated different slopes (**Figure 7**). A stratified univariate analysis was performed for the association of PI with PWT at each level of post-exercise ABI (**Table 4**). Within the "High" category, a 1 S.D. increase in PI was associated with a 27.5% increase in PWT (95% CI, 7.01 - 51.9%; R^2=0.211, *P*=0.008); however, within the "Low" category, no association was observed between PI and PWT (7.91%; 95% CI, -11.7 - 31.8%; R^2=0.020, *P*=0.443). Although suggested by the scatter plot and the stratified analysis, the interaction of PI and post-exercise ABI group was not statistically significant (*P*=0.242).

Assessing interaction between previous lower extremity vascular intervention and perfusion index

The analysis of covariance scatter plot for PI and PWT, stratified by previous vascular intervention, demonstrated intersecting slopes (**Figure 8**). A stratified univariate analysis was performed for the association of PI with PWT based on the presence or absence of previous lower extremity vascular intervention (**Table 4**). In subjects without a history of vascular intervention, a 1 S.D. increase in PI was associated with a 23.3% increase in PWT (95% CI, 0.86 - 50.8%; R^2=0.105, *P*=0.042); however, in subjects with

a history of vascular intervention, no association was observed between PI and PWT (10.2%; 95% CI, -18.7 - 49.3%; R^2=0.022, *P*=0.512). Although suggested by the scatter plot and the stratified analysis, the interaction of PI and prior vascular intervention was not statistically significant (*P*=0.681).

DISCUSSION

The primary aim of the current study was to assess the association between perfusion index (PI) and the severity of peripheral artery disease (PAD), measured as peak walking time (PWT). We found that PI is significantly associated with PWT. Of the other potential predictors of PAD severity, only pre- and post-exercise ankle-brachial index (ABI) were also associated with PWT. However, despite the fact that ABI and PI were both associated with PWT, ABI and PI were not correlated with each other. Further analysis of the relationship between these three variables showed that both ABI (either pre- or post-exercise) and PI were independent predictors of PWT. We also assessed three potential interacting variables: pre-exercise ABI, post-exercise ABI, and previous lower extremity vascular intervention. Graphical analyses suggested that the linear association between PI and PWT was dependent on the level of (pre- and post-exercise) ABI and the history of lower extremity vascular intervention. Stratified analyses showed that PI was correlated with PWT only in subjects with ABI level that was greater than the median value and in subjects without a history of lower extremity vascular intervention, whereas there was no relationship in subjects with low ABI and those with previous surgery.

First-pass gadolinium-enhanced MR perfusion of the lower limbs is a relatively novel technique for objectively measuring calf muscle perfusion, specifically in patients with PAD. For all of its benefits, ABI is still an estimate of blood flow to the extremities, and its utility in assessing tissue perfusion relies upon assumptions about the vasculature that may not be relevant in patients with PAD. When extensive collateral circulation systems develop, the amount of tissue perfusion (and, subsequently, the degree of

symptoms) may be discordant from the ABI measurement. MR perfusion offers the ability to more directly and objectively measure muscle perfusion. In a small cohort of subjects, Isbell et al established that PI could be used to distinguish individuals with PAD from normal subjects, but they did not explore the relationship between severity of disease, PI and the traditionally-used measure of ABI (9).

Because both ABI and PI approximate lower extremity blood flow, and because decreased blood flow results in claudication and decreased treadmill walk times, we expected that ABI and PI would be significantly associated with PWT. Additionally, we expected that ABI and PI would be associated with each other, however, that was not the case. Physiologically, it is reasonable to assume that muscle perfusion may not be associated with blood flow, as measured by ABI. In the setting of a rich collateral vascular network, muscle perfusion may be paradoxically elevated in the setting of a very low ABI. The lack of association between ABI and PI may be related to measurement bias. In the calculation of PI, the arterial input function is required to normalize the muscle perfusion. If we assume two individuals have the same muscle perfusion, the one with a lower arterial input function will have a higher PI. We may also be underpowered to observe the association between PI and ABI, as this specific relationship was not used in the power analysis and sample size calculations.

By analyzing the added predictive benefit of PI in addition to ABI, we gain some insight into the relationship between these two measurements and how they relate to PAD severity. From the partial F-test, we found that both PI and ABI (pre- or post-exercise) were independent predictors of PWT. In fact, when analyzing the R^2 values of the univariate and multivariate models, we see that the R^2 of the multivariate model is almost

exactly equal to the sum of the R^2 values from the univariate models. This finding suggests that PI and ABI are independent variables and may measure two different phenomena. If there were some redundancy in PI and ABI, we would expect the variance explained by the multivariate model to be less than the sum of the two individual models. If PI and ABI were synergistic, the amount of variance explained by the multivariate model should be greater than the sum of the two individual models. The finding of essentially complete independence between PI and ABI may be a function of the population studied, as one would expect that ABI and PI should be at least marginally related. By studying a population with high disease burden, we may be selecting individuals in whom ABI may not be a reliable measure and who may have extensive collateral circulation that increases muscle perfusion (7, 21). This independence may also be explained physiologically, as PI should measure the contributions from collateral microcirculation, whereas ABI will not.

Our decision to assess interaction was motivated by the literature and intuition. There is evidence that ABI may be less sensitive for disease severity in PAD at higher levels (7), and PI may be a better tool for assessing PAD in those individuals with subtle, near-normal disease. Also, because calculation of PI requires defining regions of interest in the lower extremity arterial tree, using the measurement in individuals with a history of vascular intervention may yield inaccurate data; at the very least, PI may offer no benefit over ABI in individuals with lower extremity vascular intervention. Although qualitative assessment suggested interaction was present, interaction was not statistically significant. Given our findings, we believe that interaction may exist, but that we were underpowered to observe significant interaction. The reason for PI not correlating with PWT at “Low”

ABI may be because at low arterial perfusion states, the variability of muscle perfusion is greater and thus less reliable as a predictor for PAD disease severity. The correlation between large-vessel flow and small-vessel collateralization may be widely discordant in some individuals, thus leading to poorly predictable muscle perfusion measurements. Finally, at such low perfusion, PI may not be a meaningful predictor of PAD severity over ABI alone.

Limitations to this study include the selection of the study population. Because this is a subset of a phase II randomized clinical trial, the inclusion and exclusion criteria were narrow and limiting; further, primary recruitment at the Atlanta VA led to a male-predominant cohort. Findings from this study lack generalizability to the overall population, specifically women and individuals with near-normal or normal ABI. Recruiting from a broader patient pool, including individuals without PAD, would provide greater evidence for the utility of PI as a tool for measuring PAD severity. It would also be possible to perform sensitivity and specificity analyses for PI cutoff values; currently, we are unable to provide any clinical context to a change in the value of PI. By analyzing individuals with and without PAD, we could gain even more insight into the objective value of the measurement. Because the sample size calculation was carried out for the purpose of detecting differences within the context of the clinical trial, we were likely underpowered to detect some components of the analysis, specifically interaction. This was further compromised by the fact that 17% of subjects receiving the MR perfusion protocol had uninterpretable scans. While trends exist that suggest interaction is present, a power and sample size calculation specifically conducted to assess interaction could confirm our findings. We have commented on the possible measurement bias

inherent in the calculation of PI; in addition, we were unable to control for the varying degrees of work that subjects exerted during the exercise portion of the perfusion protocol. The primary aim of using the MR perfusion protocol to measure muscle perfusion in a post-exercise patient is to mimic, as much as possible, the environment of claudication. While subjects were instructed to use the foot pedal ergometer until exhaustion, it would still be ideal to quantify the amount of work performed by the individual for use in the analysis.

Overall, this study represents the largest single cohort of PAD subjects to date to receive the MR perfusion protocol and suggests that PI could be a valuable tool for the assessment of PAD disease severity, specifically for individuals with a higher, near-normal ABI and no history of lower extremity vascular intervention. Additionally, PI is an independent predictor of PAD severity and provides additional information over ABI alone. Further study with a larger, more generalized population, enhanced imaging techniques, and objective measurement of pre-perfusion study work via the foot pedal ergometer is warranted to better elucidate the associations between PI and PWT.

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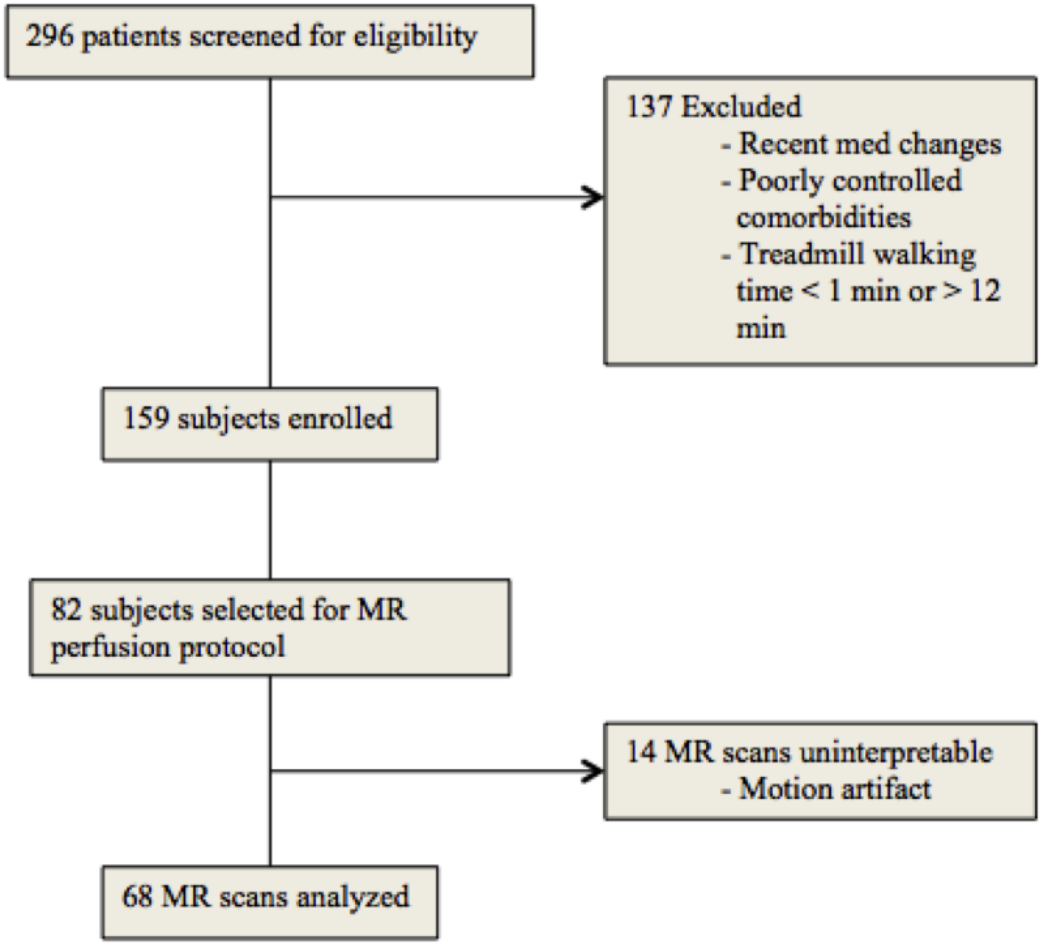
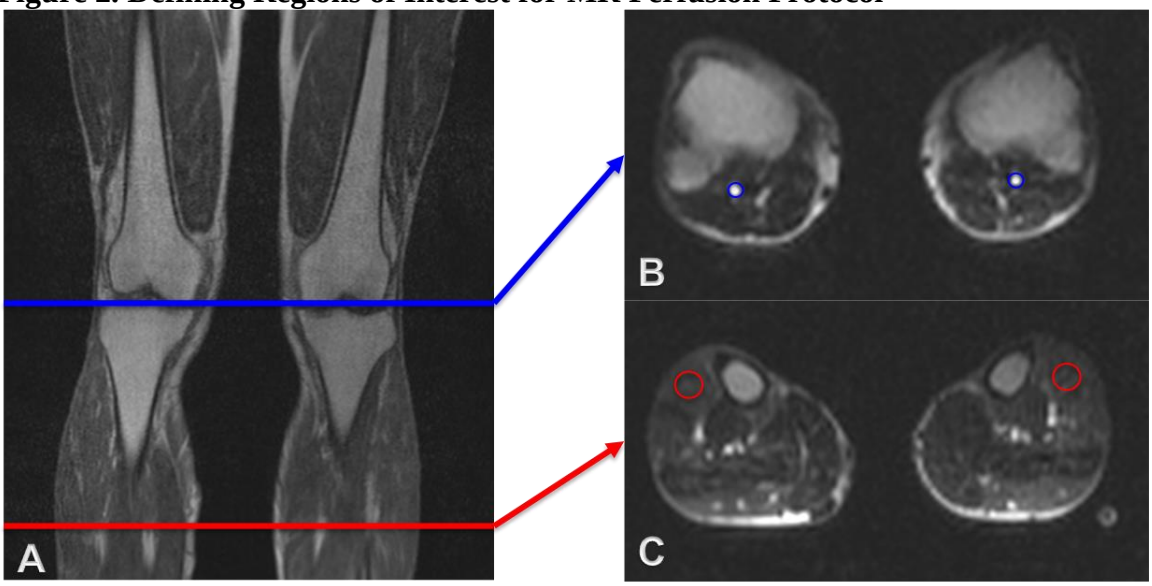
Figure 1. Selection of Subjects for Participation in the Magnetic Resolution (MR) Perfusion Protocol Arm of the GPAD-2 Study



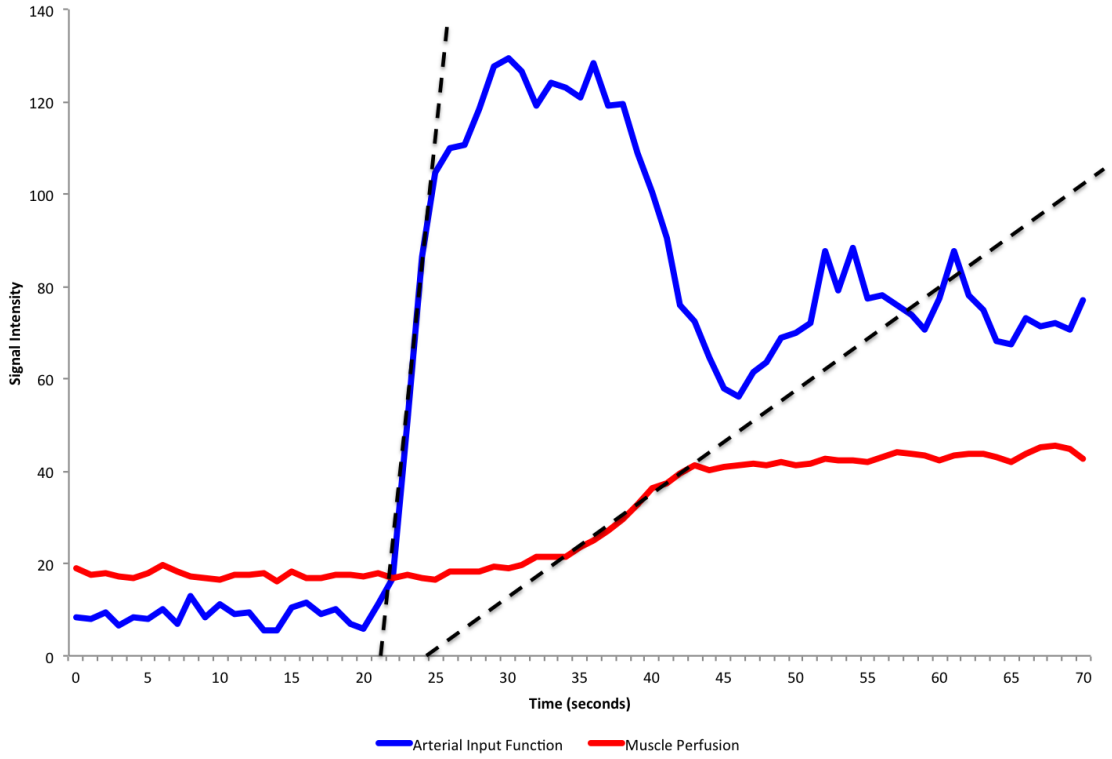
GPAD-2 indicates Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and Mobilization of Progenitor Cells in Peripheral Arterial Disease.

Figure 2. Defining Regions of Interest for MR Perfusion Protocol



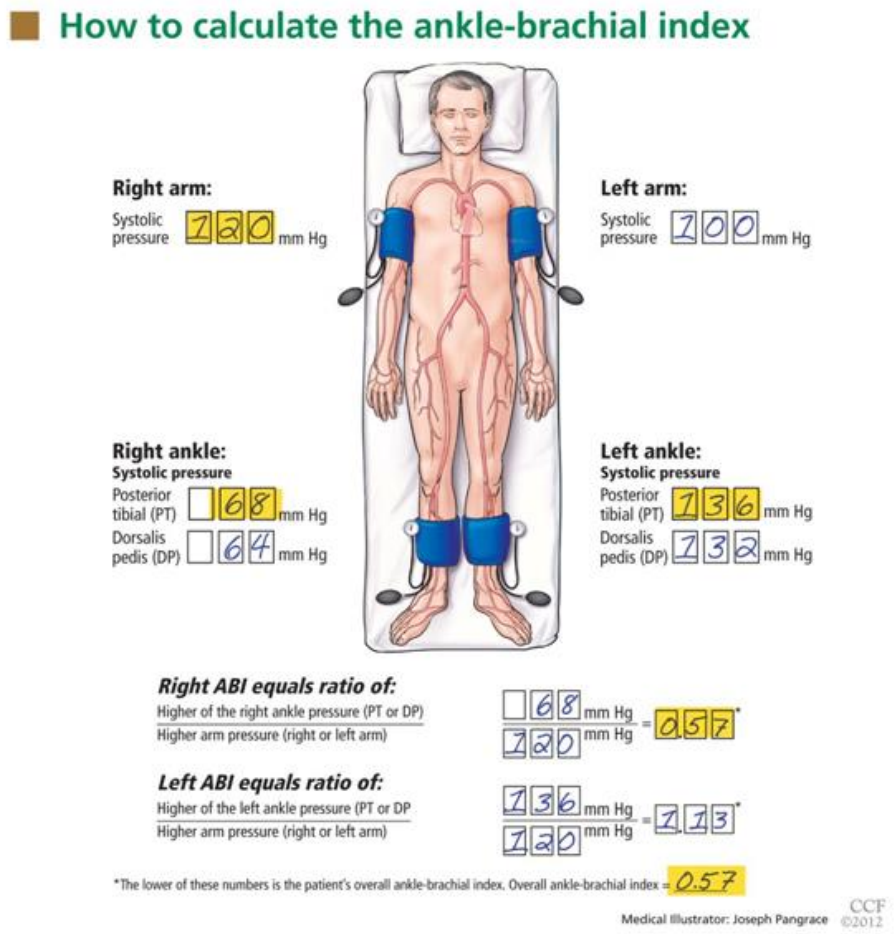
The scout image (A) of the bilateral lower extremities is shown in the coronal plane. The blue line corresponds to the level of the popliteal artery, and the red line corresponds to the level of the calf muscle. Regions of interest (ROIs) are defined to measure changes in signal intensity for the arterial input function (B, blue circles) and muscle perfusion (C, red circles).

Figure 3. Calculation of the Perfusion Index



Time-intensity curves (TICs) from regions of interest (ROIs) in the popliteal artery (blue) and the calf muscle (red) are created by measuring signal intensity over time following infusion of gadolinium. The arterial input function (AIF) and the muscle perfusion (MP) are calculated as the peak upslope (dashed line) in the blue and red TICs, respectively.

Figure 4. Calculation of the Ankle-Brachial Index



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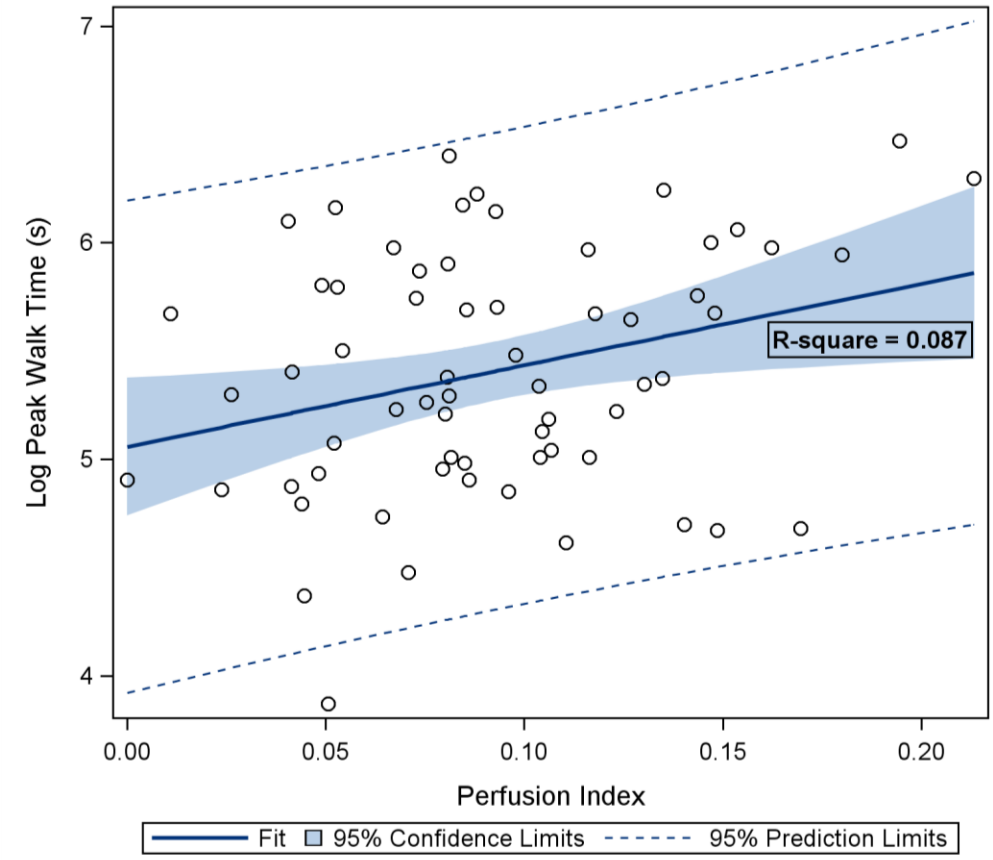
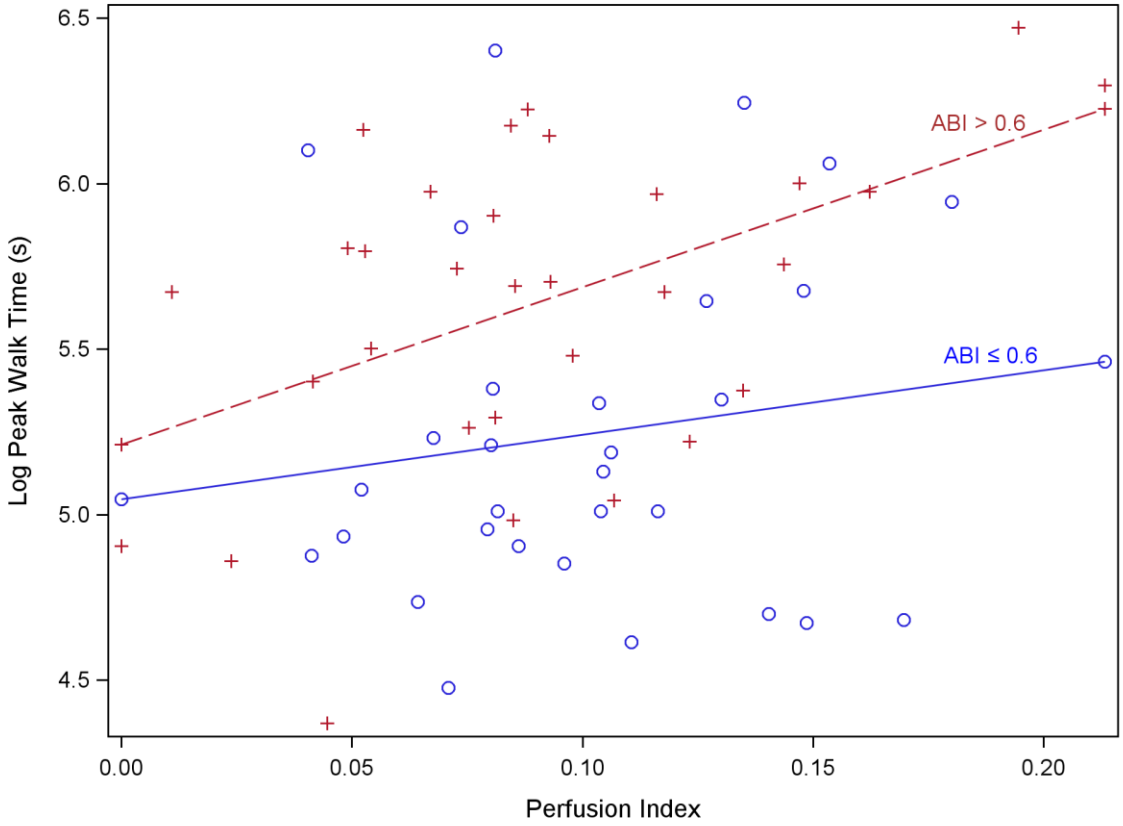
With the patient supine and at rest for 10 minutes, blood pressure cuffs are placed on all four limbs, while ensuring that the arms and ankles are level with the heart. Blood pressure is taken with a handheld Doppler device. To calculate the ankle-brachial index, the higher of the two arm pressures are used as the denominator, while the higher of the two ankle pressures (either posterior tibial or dorsalis pedis) is used as the numerator. An ABI < 0.9 in either lower extremity confirms the diagnosis of peripheral arterial disease. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2012-2013. All Rights Reserved.

Figure 5. Linear association between Perfusion Index and Peak Walk Time



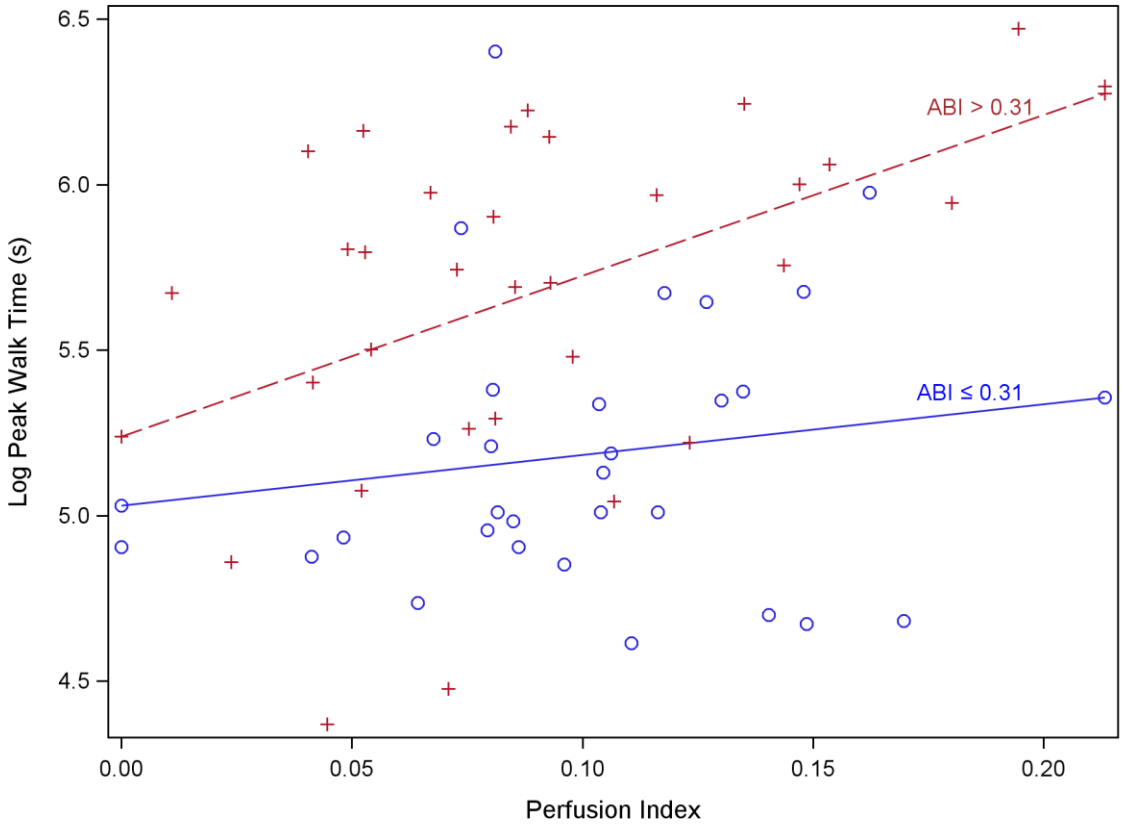
In an unadjusted linear regression, perfusion index correlates with peak walk time (P=0.018).

Figure 6. Linear Association Between Perfusion Index and Peak Walk Time, Stratified on Level of Pre-Exercise Ankle-Brachial Index



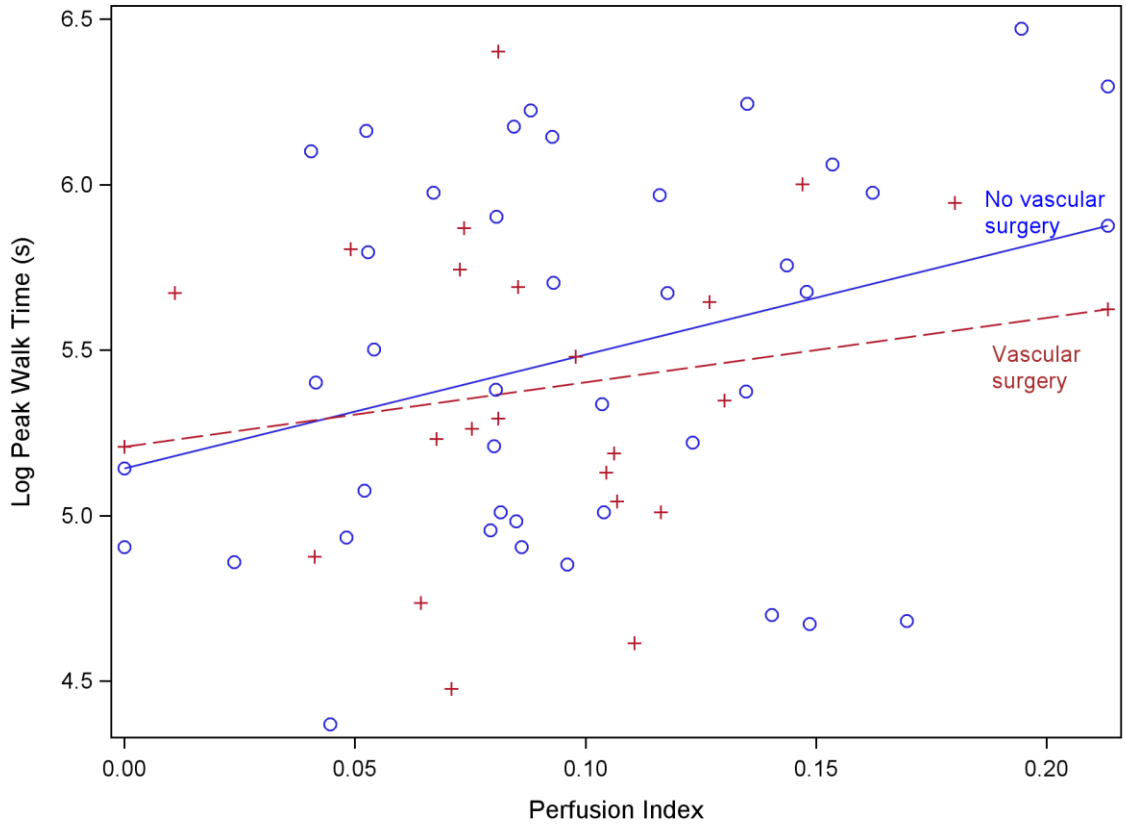
In the "High" pre-exercise ABI group (red crosses), the relationship between perfusion index and peak walking time show a greater positive association (dashed red trendline) than in the "Low" pre-exercise ABI group (blue circles, solid blue trendline). The interaction term between perfusion index and level of pre-exercise ABI is non-significant (*P*=0.345).

Figure 7. Linear Association Between Perfusion Index and Peak Walk Time, Stratified on Level of Post-Exercise Ankle-Brachial Index



In the "High" post-exercise ABI group (red crosses), the relationship between perfusion index and peak walking time show a greater positive association (dashed red trendline) than in the "Low" post-exercise ABI group (blue circles, solid blue trendline). The interaction term between perfusion index and level of post-exercise ABI is non-significant (*P*=0.242).

Figure 8. Linear Association Between Perfusion Index and Peak Walk Time, Stratified on Status of Previous Lower Extremity Vascular Intervention



In the non-lower extremity vascular intervention group (blue circles), the relationship between perfusion index and peak walking time show a greater positive association (solid blue trendline) than in the lower extremity vascular intervention group (red crosses, dashed red trendline). The interaction term between perfusion index and lower extremity vascular intervention status is non-significant (P=0.681).

Table 1. Baseline Clinical Characteristics of the Study Population

Subject characteristics	**N**	**Value**
Mean age, years ± SD	82	63.9 ± 8.2
Males (%)	82	74 (90.2)
Caucasian race (%)	82	34 (41.5)
Smoking, pack-years ± SD	82	38.9 ± 12.9
Diabetes (%)	82	26 (31.7)
Hypertension (%)	81	78 (96.3)
Hyperlipidemia (%)	81	68 (84.0)
Myocardial infarction (%)	82	20 (24.4)
Stroke (%)	82	17 (20.4)
Lower extremity surgical intervention (%)	78	29 (37.2)
Ankle-brachial index ± SD Pre-exercise Post-exercise	 77 77	 0.61 ± 0.14 0.38 ± 0.20
Perfusion index ± SD	68	0.09 ± 0.05
Peak walking time, sec (IQR)	78	257 (150-332)

Data are expressed as the mean value ± SD, median value (IQR), or number (%) of subjects.

Table 2. Univariate Predictors Peak Walking Time

Variable	**% Change in PWT per unit (95% CI)**	**R^2**	***P*-value**
Perfusion index (0.05 Δ)	20.7 (3.42 – 40.9)	0.087	0.018
Pre-exercise ABI (0.1 pt. Δ)	19.5 (10.1 – 29.1)	0.237	<0.0001
Post-exercise ABI (0.1 pt. Δ)	15.4 (8.49 – 22.8)	0.260	<0.0001
Age (10 yr. Δ)	-6.36 (-22.5 – 13.1)	0.008	0.489
Caucasian vs. any other race	-24.1 (-42.7 – 0.40)	0.059	0.054
Male vs. female	1.38 (-37.9 – 65.5)	0.000	0.956
Smoking (10 pack-year Δ)	-10.0 (-19.1 – 0.13)	0.061	0.053
Diabetes (Y vs. N)	-3.38 (-28.7 – 30.9)	0.001	0.822
Hypertension (Y vs. N)	-12.3 (-55.4 – 67.2)	0.002	0.699
Hyperlipidemia (Y vs. N)	3.72 (-30.0 – 53.7)	0.001	0.853
Myocardial infarction (Y vs. N)	-9.34 (-36.4 – 29.2)	0.005	0.582
Stroke (Y vs. N)	11.2 (-23.8 – 62.3)	0.005	0.576
Lower extremity vascular intervention (Y vs. N)	-5.20 (-30.1 – 28.7)	0.002	0.728

Abbreviations: Δ = increase in the stated interval; ABI = ankle-brachial index; PWT = peak walking time

PWT was log-transformed for normality, and all parameter estimates are expressed in the percent change in PWT. Analyses performed using a generalized linear model: linear regression for continuous variables, ANOVA for categorical variables.

Table 3. Assessing the Value of Perfusion Index, Given Ankle-Brachial Index, in Predicting Peak Walking Time using the Partial F-Test

Model	**R^2**	**Partial F-test statistic**	***P*-value**
PI	0.087	--	--
Pre-Exercise ABI			
ABI	0.237	8.91	0.004
ABI + PI	0.335		
Post-Exercise ABI			
ABI	0.260	11.76	0.001
ABI + PI	0.381		

Abbreviations: PI = perfusion index; ABI = ankle-brachial index; PWT = peak walking time

Outcome variable models is the log-transformed PWT. Partial F-test statistic is comparing the full model (with PI) to the reduced model (ABI alone). *P*-value is for the significance of the F-test statistic. $P<0.05$ indicates that PI adds to the prediction of PWT, given ABI is in the model.

Table 4. Association of Peak Walking Time and Perfusion Index, Stratifying on Level of Ankle-Brachial Index and Previous Lower Extremity Vascular Intervention

Stratified Variable	**% Change in PWT per 1 SD increase in PI (95% CI)**	**R^2**	***P*-value**
Pre-Exercise ABI			
ABI ≤ 0.6 (N=30)	10.2 (-15.5 – 43.7)	0.020	0.379
ABI > 0.6 (N=33)	29.3 (10.5 – 51.3)	0.263	0.004
Post-Exercise ABI			
ABI ≤ 0.31 (N=31)	7.91 (-11.7 – 31.8)	0.020	0.443
ABI > 0.31 (N=32)	27.5 (7.01 – 51.9)	0.211	0.008
Previous Vascular Intervention			
Yes (N =22)	10.2 (-18.7 – 49.3)	0.022	0.512
No (N=40)	23.3 (0.86 – 50.8)	0.105	0.042

Abbreviations: PWT = peak walking time; SD = standard deviation; PI = perfusion index; ABI = ankle-brachial index
The SD of PI is 0.05.

APPENDIX A: **Criteria for participation in the GPAD-2 study**

Inclusion

- Males or females between 21 and 80 years of age
- Documented PAD (by ABI or angiography)
- Clinically stable (at least 2 months) history of intermittent claudication with no change in symptom severity in the 2 months prior to screening
- On stable statin therapy for previous 3 months
- Peak walking time (PWT) between 1 and 12 minutes on a standardized Gardner treadmill protocol
- A Doppler-derived ABI < 0.85 in the symptomatic limb after 10 minutes of rest at screening. For subjects with an ABI > 1.3 (non-compressible arteries), a toe-brachial index (TBI) < 0.70 must be obtained for subject qualification, or if ABI is > 0.85 to 1.0, a reduction of 20% in ABI measured within 1 minute of treadmill testing must be obtained for subject qualification
- On appropriate and stable medical therapy for atherosclerosis for at least 2 months
- If diabetic, the subject should have a dilated eye exam excluding proliferative retinopathy in the previous 12 months

Exclusion

- Recent or current active infections (treated with antibiotics)
- Recent (3 months) change in statin therapy
- Critical limb ischemia either chronic (category 3 and 4 of SVS classification) or acute ischemia manifested by rest pain, ulceration, or gangrene
- Lower extremity vascular surgery, angioplasty or lumbar sympathectomy within 3 months of enrollment
- Participation in a structured exercise treatment protocol within 3 months of enrollment
- Severe heart failure (NYHA class III or IV), heart muscle disease or atrial fibrillation

- Limitation on exercise for symptoms other than intermittent claudication such as arthritis or dyspnea
- Uncontrolled diabetes mellitus (defined as HbA1c > 10.0)
- Chronic renal disease (creatinine of > 2.5 mg/dl) or hepatic disease (> 3 X elevations in AST and ALT)
- Ophthalmologic conditions associated with a neo-vascular response
- Alcohol or drug abuse, or any other disease process that, in the opinion of the PI, will interfere with the ability of the patient to participate in the study