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Peripheral Arterial Disease Assessment *Wall, Perfusion, and Spectroscopy*

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Abstract

Introduction—Peripheral arterial disease (PAD) is characterized by lower limb arterial obstruction due to atherosclerosis and is increasingly common. Presently used methods for diagnosis and follow-up as well as for assessment of novel therapies are limited.

Materials and Methods—Three distinct magnetic resonance examinations were developed. The first was high-resolution black-blood atherosclerotic plaque imaging of the superficial femoral artery using a surface coil and flow saturation. Second, first-pass contrast-enhanced dual-contrast perfusion imaging of the calf muscle was performed at peak exercise using a magnetic resonance (MR)–compatible pedal ergometer. Lastly, ^{31}P MR spectroscopy was also performed at peak exercise to measure phosphocreatine (PCr) recovery kinetics.

Results—Seventeen patients (age, 63 ± 10 yrs) with mild to moderate PAD were studied with black-blood atherosclerotic plaque imaging. Mean atherosclerotic plaque volume measured was $7.27 \pm 3.73 \text{ cm}^3$. Eleven patients (age, 61 ± 11 yrs) with mild to moderate symptomatic PAD and 22 normal control subjects were studied with first-pass contrast-enhanced perfusion imaging. Perfusion index was stepwise increased from patients to normal subjects with matched workload to normal subjects at maximal exercise. For PCr recovery kinetics, 20 patients with mild to moderate PAD and 14 controls were studied. The median recovery time constant of PCr was 34.7 seconds in the controls and 91.0 seconds in the PAD patients ($P < 0.0001$).

Conclusions—Three distinct MR examinations of different aspects of peripheral arterial disease have been developed and tested and shown to differentiate patients with mild to moderate PAD from normal controls. Taken together, these tests are potential quantitative end points for clinical trials of novel therapies in PAD.

Keywords

magnetic resonance imaging; peripheral arterial disease; atherosclerotic plaque; muscle perfusion; spectroscopy; phosphocreatine

Peripheral arterial disease (PAD) is characterized by lower limb arterial obstruction due to atherosclerosis. There are more than 10 million individuals in the United States with this disease, most of whom are elderly.¹ In the general population, age-adjusted prevalence is approximately 12%.² The incidence in those older than 70 years is more than 20%, and the disease is becoming more prevalent with the advancing age of the US population.³ More recent data suggest that in populations at risk, including persons with diabetes or cigarette smokers,

the incidence of PAD is nearly 30%.⁴ Nearly half of these had no evidence of other cardiovascular disease.

Presently used diagnostic methods include the ankle-brachial index (ABI),⁵ duplex ultrasonography,⁶ venous plethysmography,⁷ and angiography by x-ray or magnetic resonance (MRA),⁸ all of which have limitations. The ABI screening is limited in calcified vessels, aortoiliac disease, collateralized disease, and occlusions. Plethysmography cannot provide spatial localization of flow, may underestimate perfusion, and can be sensitive to changes in both body position and temperature.⁹ Artifacts in MRA that are potential challenges to its diagnostic accuracy include signal loss from in-plane saturation, turbulent flow, and metallic clips and stents.¹⁰ X-ray angiography and MRA image only the lumen of the vessel, not the wall, and do not assess end-organ effects of PAD. Diagnostic modalities that will allow study of progression and regression of disease are clearly needed.

With its flexibility, spatial resolution, and ability to assess metabolic parameters, combined MR imaging (MRI) and spectroscopy can be ideal for the comprehensive noninvasive assessment of PAD. To assess the burden of atherosclerosis in PAD, one approach would be to measure atherosclerotic plaque volume (APV) and composition using black-blood vessel wall imaging. Knowing plaque burden alone is not enough because it is also important to measure the effects of atherosclerosis on skeletal muscle function. A noninvasive technique capable of measuring tissue perfusion would be of great clinical value because improving blood flow is a major therapeutic goal in patients with PAD. In addition, phosphocreatine recovery after exercise as measured by phosphorus-31 magnetic resonance spectroscopy (³¹P MRS) may be an important parameter in patients with PAD and may be measuring more than just abnormal blood flow.^{11,12} By relying on both the arterial oxygen supply and the tissues' capacity to use substrate, measurements of PCr regeneration incorporate many of the adaptive and/or maladaptive cellular changes developed in response to a chronic limitation in blood flow.¹³ Our group has performed a series of studies to validate and assess the potential use of these measures in PAD.^{14–16}

Materials and Methods

Superficial Femoral Artery Plaque Volume Measurements

Study Protocol—Patients between the ages of 30 and 85 years with symptoms of intermittent claudication without critical limb ischemia and an ABI between 0.4 and 0.9 were eligible to enroll in the study of atherosclerotic plaque imaging in the superficial femoral artery. All subjects were placed supine in a 1.5-T Avanto or Sonata MR scanner (Siemens Medical Solutions, Erlangen, Germany) with the calf at the isocenter of the magnet. A custom-built flexible, linear, 4-element (10 × 10 cm² element) surface coil array (Nova Medical, Wilmington, Mass) was placed over the thigh and superficial femoral artery (SFA). A multislice turbo-spin-echo pulse sequence with fat presaturation was used. Flowing blood was suppressed using spatial presaturation, with a combination of periodic excitation of upstream slices and spatial presaturation. Additional flow suppression for downstream slices was provided by the imaging excitations themselves so that blood was suppressed throughout the multislice data set. Other imaging parameters included repetition time, 1100 milliseconds; echo time, 7.6 milliseconds; echo spacing, 7.5 milliseconds; turbo factor, 9; voxel size, 0.5 × 0.5 × 3 mm; 4 signal averages, with interleaved image sets used to cover the length of the SFA. Imaging began above the femoral bifurcation and continued as far as possible through the adductor canal. Representative contiguous images of the femoral artery from a patient with PAD are shown (Fig. 1).

Image Analysis—Before quantification of plaque volume, image quality was assessed and deemed adequate or inadequate for analysis. A total of 23 patients were imaged, and 17 had

image quality suitable for analysis. In 4 of the 6 excluded data sets, complete occlusion of the SFA precluded analysis. Occlusion of flow was verified with velocity-encoded imaging. In 1 subject, obesity (body mass index [BMI], 33) and a metal artifact precluded analysis, and in another subject, obesity (BMI >42) led to poor image quality.

In those subjects with a patent SFA and adequate image quality, APV of the SFA was contoured by 2 independent operators using VesselMASS software (University of Leiden, Leiden, The Netherlands). For each individual slice, both the luminal and adventitial borders were manually delineated and cross-sectional area of the vessel wall measured. Total vessel wall volume was calculated as

$$\text{Volume} = \sum_{i=1}^n \text{CSA}_i \times H$$

where H is the slice thickness, CSA is the cross-sectional area, and n is the number of slices in the 3-dimensional data set. Volume measurements were initiated at the bifurcation of the common femoral artery, and total vessel distance covered for each analysis was predefined. Nine patients returned (at a mean of 24 days) for evaluation of test-retest reliability using anatomical landmarks and matched number of acquired slices. Imaging time was measured from acquisition of the first scouts to completion of the imaging protocol but does not include time for patient positioning, coil placement, and insertion and removal from the scanner.

First-Pass Contrast-Enhanced Calf Muscle Perfusion at Peak Exercise

Study Protocol—Patients between the ages of 30 and 85 years with symptoms of intermittent claudication without critical limb ischemia and an ABI between 0.4 and 0.9 were eligible for this study. Eleven patients (age 61 ± 11 years) were recruited. Normal human subjects without risk factors for atherosclerosis were recruited from the community to serve as controls.

All subjects were placed supine in the Avanto or Sonata 1.5-T MR scanner with the calf at the isocenter of the magnet. Monitoring of the electrocardiogram and blood pressure was performed with an InVivo 3155MVS monitor (Intermetrics Companies, Orlando, Fla) throughout the study. An MRI-compatible plantar-flexion exercise ergometer (Lode, The Netherlands) constructed for use in this protocol was then affixed to the MR table. The subjects' feet were strapped into the independently operating pedal shoes, and a rectangular circularly polarized 20×50 -cm flexible receive coil was wrapped around the calf of interest. The subjects were then placed into the magnet bore with the calf at the isocenter, and scout images were acquired. The maximal exercise group (NI_{max} ; $n = 11$; age, 46 ± 10 years) and PAD were then instructed to push against the pedal at a steady rate (10–12 rpm) until exhaustion or limiting symptoms developed, exercising the muscles of the foot and calf. The normal, low-level exercise group (NI_{low} ; $n = 11$; age, 52 ± 6 yrs) was studied at a later time point and instructed to exercise to a prespecified workload, matched to that achieved by the PAD patients. Immediately after the cessation of exercise, 0.1 mm kg^{-1} of gadopentatate dimeglumine (Berlex, Montville, NJ) was infused at 3 to 4 mL s^{-1} through a large-bore IV placed in the antecubital vein followed by a 20-mL saline flush also infused at 3 to 4 mL s^{-1} . Imaging began simultaneously immediately after exercise cessation and continued for 100 acquisitions.

Imaging Technique—The dual-contrast sequence consisted of 2 interleaved slices positioned 5 cm apart with the lowest slice centered in the widest part of the calf (Fig. 2). A spoiled gradient echo (GRE) pulse sequence was modified to accommodate the simultaneous acquisition of muscle perfusion and the arterial input function (AIF). An interleaved acquisition was performed where saturation recovery (SR) GRE (inversion time [TI] = 10 ms) was used

to image the AIF and inversion recovery (IR) GRE (TI = 320 ms) was used for muscle imaging. The delay between successive excitation pulses of the SR and IR sequences was 3.6 milliseconds. The TI times specify the delay between the preparation pulses and the start of the data readout. The SR and IR radiofrequency pulses were nonselective. The center of k-space was measured in the first excitation pulse period (centric k-space order), and the preparation pulses were applied via the body coil. Flow compensation was used to reduce arterial flow artifact.

This pulse sequence design was used to maximize sensitivity to contrast enhancement in the muscle while avoiding saturation of the AIF signal.¹⁷ Other parameters for both sequences included field of view, 180 × 180; matrix, 64 × 64; flip angle, 15 degrees; repetition time, 900 milliseconds, echo time, 1.86 milliseconds; receive bandwidth, 625 Hz per pixel; and repetitions, 100. Electrocardiogram gating was not used to maintain a constant repetition time, although this choice does make the method more sensitive to blood inflow effects, including pulsatile flow artifact and variations in the AIF signal. Time-intensity curves (TICs) were generated for regions of interest drawn in the muscle and input arteries (Fig. 2). A perfusion index (PI) was defined as the slope of the tissue TIC divided by the slope of the AIF TIC.

Image Analysis—Data were analyzed using ARGUS image analysis software (Siemens). For the muscle TIC, a single operator defined at least 3 regions of interest approximately 1.5 cm² in separate muscles of the calf, with care taken to avoid the vasculature. To generate each TIC, the mean signal intensity was used. Because of heterogeneous nature of blood flow to the calf depending on the muscles used in exercise, the muscle region with the greatest signal intensity by visual inspection during the first pass was used for the analysis. For the AIF, the SR slices were analyzed. A smaller region of interest was used (0.3–0.4 cm²) in the vessels to avoid contamination with tissue signal, and the TIC with the greatest upslope was used. Both foot and peak of TICs were established using a linear fit model. No coil-baseline correction was used. Twenty-two randomly selected data sets were analyzed for intraobserver variability.

Phosphocreatine Recovery Kinetics After Exercise

Study Protocol—Patients between the ages of 30 and 85 years with symptoms of intermittent claudication without critical limb ischemia and an ABI between 0.4 and 0.9 were eligible for this study. Normal human subjects without risk factors for atherosclerosis were recruited from the community to serve as controls. The protocol was approved by the Human Investigation Committee at the University of Virginia, and all participants signed informed consent.

³¹P spectra were acquired with the Siemens Sonata 1.5-T MR scanner using a single-pulse, surface coil–localized, 512-millisecond free induction decay acquisition, with the coil centered on the midcalf. A standard ³¹P surface coil in the patient table was used. A coil signal intensity profile with a phosphorus phantom demonstrated excellent signal within 7 to 8 cm. This distance fully encompasses the gastrocnemius and soleus of the average patient's calf, the muscles worked during plantar flexion.

Siemens spectroscopy software on a Leonardo workstation (Siemens Medical Solutions, Malvern, Pa) was used to estimate relative concentrations of adenosine triphosphate (ATP), PCr, and Pi. Free induction decays were multiplied by an exponential with a time constant of 110 milliseconds and zero-filled from 1024 to 2048 points before Fourier transformation. Phase and baseline corrections were made, followed by Lorentzian fitting and integration of the spectral peaks. The PCr recovery time constant (*R*), which characterizes the PCr recovery time, was calculated by monoexponential fit of the PCr levels versus time (beginning at the end of exercise) using the equation $y = a(b - e^{-t/R})$, where *y* is the fitted PCr level, *t* is the time after cessation of exercise, and *a* and *b* are the fitted relative levels of the PCr recovery curve. Tissue pH was calculated from the chemical shift between Pi and PCr (δ) using the following formula:

$$\text{pH} = 6.77 + \log_{10}((\delta - 3.29)/(5.68 - \delta)).$$

All subjects were placed supine in the MR scanner with the calf at the magnet isocenter. Monitoring of the electrocardiogram and blood pressure was performed with an InVivo 3155MVS (Intermagetics). After shimming to minimize nonuniformity of the magnetic field, 5 baseline spectra were acquired. An MRI-compatible plantar-flexion exercise device was then affixed to the MR table. The subject was instructed to push the pedal at a steady rate until exhaustion or limiting symptoms developed, exercising the calf muscles. After 4 preparation pulses, 25 signal averages at a repetition time of 550 milliseconds were acquired for a total acquisition time of 16 seconds per spectrum. Two spectra were acquired before the end of exercise, followed by the acquisition of 18 spectra commencing at the end of exercise. Three PAD patients and 8 normal subjects returned at 315 ± 188 days after initial study for studies of test-retest reliability.

Statistical Analysis

For all study parameters, subject characteristics were summarized as mean and SD for continuous variables and by frequencies for other variables. Subject characteristics were compared between controls, and patients using *t* tests and were summarized as mean and SD. The intraclass correlation coefficients of reliability were calculated for interobserver, intraobserver, and reproducibility data using the output of analysis of variance from SAS statistical software (SAS 9.0; Cary, NC). Reproducibility and both interobserver and intraobserver variability were also analyzed using the method of Bland and Altman.¹⁸ MedCalc software (Belgium, version 8.1.0.0) was used to generate Bland-Altman plots.

The Wilcoxon signed rank sum test was used to compare the centers of the PI and total work for PAD, NI_{max} and NI_{low} and exercise times, PCr recovery time constant, pH, and PCr/ATP, which were then summarized as median, 25th percentile, and 75th percentile. A binormal receiver operating characteristic (ROC) curve was estimated using MedCalc statistical software. For comparison of intraobserver variability, an intraclass correlation coefficient was calculated and measurements of agreement compared using the method of Bland and Altman.¹⁸ Statistical analyses were performed in Splus 2000 Professional Release 2 (MathSoft, Inc).

Using the test-retest reliability data of APV data, we estimated the sample sizes needed to detect the true difference between the means of APV with both 80% and 90% power. Assuming a 2-tailed test, a significance level of 0.05, and using the estimated standard deviation, the sample sizes needed to detect the true mean differences ranging from 1% to 5% of the mean APV were established.

Results

SFA Plaque Volume Measurements

Seventeen patients (age 63 ± 10 yrs) with mild to moderate PAD (ABI, 0.72 ± 0.11) were studied. Of 17 patients, 10 had known coronary artery disease, 8 had diabetes mellitus, 2 had a prior cerebrovascular accident (CVA), and all were current or former smokers. Peak systolic blood pressure was 143 ± 22 , and mean BMI was 28.7 ± 10.0 . Nineteen SFA vessels in the 17 patients were evaluated (mean length, 17.1 ± 5.2 cm), with total image acquisition time of 13.1 ± 2.7 minutes per vessel. Mean APV measured was $7.27 \pm 3.73 \text{ cm}^3$.

For the interobserver data, intraclass correlation coefficient was $R = 0.987$ (95% confidence interval [CI], 0.967–0.995). By Bland-Altman analysis, the percent difference between operator measurements was $5.2\% \pm 3.3\%$ of the total plaque volume measured per patient. A

single operator performed repeat analysis of the 19 vessel segments at a mean of 24 days later. For intraobserver data, intraclass correlation coefficient was $R = 0.997$ (95% CI, 0.993–0.998). For reproducibility data, intraclass correlation coefficient was $R = 0.996$ (95% CI, 0.991–0.999). Sample size estimates shown in Table 1 are for a study evaluating APV progression/regression and used an SD of 0.27 cm^3 obtained from the test-retest reliability data. These estimates assume all patients enrolled will have adequate image quality for analysis.

First-Pass Contrast-Enhanced Calf Muscle Perfusion at Peak Exercise

Eleven patients (age, 61 ± 11 years) with mild to moderate symptomatic PAD (ABI, 0.75 ± 0.08) and 22 normal control subjects were studied. Of 11 PAD patients, 7 had previously documented coronary artery disease. Two had a prior CVA, 5 had diabetes, and all were current or former smokers. All PAD and 11 NI (NI_{max} ; age, 46 ± 10 years; $P = 0.003$ vs PAD) exercised 1 leg to exhaustion while supine in a 1.5-T MRI scanner using the custom-built, 2-pedal plantar-flexion ergometer. An additional 11 normal subjects (NI_{low} ; age, 52 ± 6 ; $P = \text{not significant}$ [NS] vs PAD) exercised submaximally to a predefined workload similar to that achieved by the PAD subjects. Median work performed was 120 J in PAD (25th and 75th percentiles are 100 and 418, respectively), with a mean of 227 and 210 J in NI_{low} (25th and 75th percentiles are 185 and 221, respectively; $P = \text{NS}$ vs PAD), with a mean of 210 and 698 J in NI_{max} (25th and 75th percentiles are 427 and 1425, respectively; $P < 0.001$ vs NI_{low} and PAD), and with a mean of 1047 (Fig. 3).

Perfusion heterogeneity in the muscles of the lower leg was visually observed in all subjects in part due to which muscles were used by that individual during exercise. The anterior tibialis had the greatest perfusion upslope in 67% of subjects and, thus, was the most frequently used muscle group for calculating PI. The gastrocnemius and soleus had the greatest upslope in 24% and 9% of subjects, respectively.

Median PI was 0.29 in PAD (25th and 75th percentiles are 0.20 and 0.40, respectively), 0.48 in NI_{low} (25th and 75th percentiles are 0.36 and 0.62, respectively; $P < 0.02$ vs PAD), and 0.69 in NI_{max} (25th and 75th percentiles are 0.51 and 0.77, respectively; $P < 0.001$ vs PAD). Perfusion index compared between the 3 groups is shown in Figure 4. Median PI was 0.57 among all control subjects (25th and 75th percentiles are 0.46 and 0.75, respectively; $P < 0.001$ vs PAD). In the PAD group, the ABI failed to correlate with PI ($P = \text{NS}$), and neither the ABI nor the PI correlated with the workload achieved ($P = \text{NS}$) based on linear regression analysis. Furthermore, PI failed to correlate with workload achieved in the NI_{max} group and when the entire normal cohort was pooled ($P = \text{NS}$). There was a weak relationship between workload and PI when data for all 3 groups were pooled ($r = 0.379$; $P < 0.03$).

To assess the ability of PI to discriminate disease defined as symptomatic PAD with an ABI less than 0.9 from no disease, ROC curves were generated. When both normal groups (NI_{max} and NI_{low}) and PAD are used to generate the ROC and using a cutoff value for PI of 0.34, the area under the curve is 0.88 (95% CI, 0.71–0.96). The area under the curve was 0.95 (95% CI, 0.77–0.99) when only PAD and NI_{max} are analyzed together. Intraobserver variability was evaluated in 22 data sets. Intraclass correlation coefficient was $R = 0.910$ for intraobserver variability.

PCr Recovery Kinetics After Exercise

Twenty patients (12 men) and 14 controls (5 men) were studied. Among PAD patients, the average ABI was 0.62 ± 0.13 . Eighty percent of patients had previously documented coronary artery disease. Thirty-two percent had a prior CVA and a similar proportion had diabetes, whereas 95% were either current or former smokers. The patients were older than the controls (67 ± 10 vs 45 ± 14 years, respectively; $P < 0.0001$). Mean systolic blood pressure of patients

was 141 ± 19 mm Hg. Eleven of the patients had undergone recent x-ray angiography, and 9 of these had complete occlusions of at least 1 vessel (6 superficial femoral, 3 posterior tibial, 2 anterior tibial, 1 iliac, and 1 common femoral) with extensive collateralization.

Median exercise time was 195.0 seconds in normal (25th percentile and 75th percentile are 161.2 and 333.8 seconds, respectively) and 162.5 seconds in PAD patients (25th and 75th percentiles are 138.8 and 183.8 seconds, respectively; $P = 0.06$). Representative recovery plots (Fig. 5) are shown. The median recovery time constant of PCr was 34.7 seconds in the controls (25th and 75th percentiles are 25.9 and 51.2 seconds, respectively) and 91.0 seconds in the PAD patients (25th and 75th percentiles are 65.0 and 134.5 seconds, respectively; $P < 0.0001$) (Fig. 6). An ROC curve was constructed to determine the discriminatory power of the PCr recovery time constant for detecting PAD. The area under the fitted curve was 0.925 ± 0.045 .

Phosphocreatine/ATP ratios were similar between the 2 groups both at rest ($P = 0.50$) and at end exercise ($P = 0.54$). The median pH measured at the completion of exercise was 7.02 in PAD and 6.95 in controls ($P = 0.19$). No significant correlation between PCr recovery time and age was detected within either subgroup ($P = 0.31$ for controls and $P = 0.56$ for PAD) or within the entire cohort ($P = 0.12$). Phosphocreatine recovery time was also not significantly correlated with exercise time, within either subgroup ($P = 0.27$ for controls, $P = 0.71$ for patients), or within the entire cohort ($P = 0.17$). No relationship was observed between PCr recovery time and ABI based on linear regression analysis ($P = 0.68$). Furthermore, exercise time did not correlate with ABI in patients ($P = 0.27$).

Clinical follow-up was completed in 100% of PAD subjects at a mean of 435 ± 93 days. One patient died of cardiovascular complications after nonvascular surgery. Six of the limbs studied required revascularization or amputation. Percutaneous revascularization was performed in 2 subjects and surgical revascularization in 4. In all but 2 cases, progressive worsening of claudication was the indication for intervention, with 1 experiencing an acute occlusion and another recurrent infection and tissue loss. Median PCr recovery was 121.0 seconds (25th and 75th percentiles are 91.8 and 220.0 seconds, respectively) in patients that died during follow-up or had limbs that required surgery, angioplasty, or amputation compared with 71.9 seconds in survivors that did not require limb intervention (25th and 75th percentiles are 51.0 and 112.8 seconds, respectively; $P = 0.057$). For PAD in the highest 75th percentile of PCr recovery kinetics (recovery time constant >118 seconds), the relative risk of subsequent surgery, angioplasty, amputation, or death was 3.11 (95% CI, 0.98–9.84). Median ABI was similar among PAD who died or had limbs that required revascularization or amputation (0.58; 25th and 75th percentiles are 0.53 and 0.65, respectively) compared with survivors free from limb intervention during follow-up (0.60; 25th and 75th percentiles are 0.52 and 0.69, respectively; $P = \text{NS}$).

Studies of test-retest reliability were performed in 8 controls and 3 PAD patients. The PCr recovery time constant was highly reproducible with an intraclass correlation coefficient $r = 0.9505$. One additional PAD subject (data not included in analysis) had undergone surgical revascularization before a repeat study performed 226 days after the initial study. In addition to a near doubling of exercise time (135 vs 230 seconds), PCr recovery time improved from 273 seconds at baseline to 60 seconds after revascularization.

Discussion

Three distinct MR examinations of different aspects of PAD have been developed and tested. We have shown that high-resolution, high-volume MR measurement of atherosclerotic plaque in the superficial femoral artery in PAD is feasible and highly reproducible and reliable. In addition, peak exercise measurement of lower limb perfusion with first-pass gadolinium-

enhanced MR can distinguish mild to moderate PAD from normal controls. Finally, ³¹P spectroscopy was used to show that PCr recovery time constant is prolonged in patients with symptomatic PAD when compared with normal individuals despite shorter exercise times. Taken together, these tests are potential quantitative end points for clinical trials of novel therapies in PAD.

SFA Plaque Volume Measurements

Potential applications of SFA plaque imaging include diagnosing preclinical vascular disease, assessing disease severity in those with established PAD, and monitoring atherosclerotic plaque progression in response to both established and novel therapies. Furthermore, this noninvasive approach can reduce required sample sizes for clinical studies using atherosclerotic plaque progression rate as a surrogate efficacy end point.

Contrast x-ray angiography remains the most commonly used clinical tool for diagnosing atherosclerosis and assessing its severity. The technique has also been used extensively in studies evaluating plaque progression rate,¹⁹ a dynamic parameter now established as an important surrogate marker of clinical risk.²⁰ However, angiography is associated with the risk of procedural complications and is hampered by its inability to visualize the vessel wall, a pitfall that can lead to systematic underestimation of plaque burden.²¹ It is now recognized that atherosclerotic plaque in coronary arteries may not encroach the lumen until the lesion occupies up to 40% of the combined arterial wall and lumen volume, a phenomenon commonly referred to as positive remodeling.²² Positive remodeling also seems to play an important role in disease progression in the peripheral vasculature,²³ suggesting that a technique capable of visualizing the vessel wall will be most accurate for the assessment of atherosclerotic disease burden in the periphery.

In recent years, intravascular ultrasound (IVUS) has emerged as the diagnostic criterion standard for imaging vessel wall pathology. Intravascular ultrasound is a catheter-based imaging technique that provides cross-sectional images of both the vessel lumen and wall with excellent spatial resolution, but it is invasive and can be associated with complications.²⁴ Although increasingly used to evaluate plaque burden and vascular remodeling as a surrogate efficacy end point in randomized clinical studies,^{20,25,26} IVUS is not an ideal strategy for making serial measurements in humans, prompting a search for novel noninvasive approaches for quantifying atherosclerotic plaque.

There are a number of advantages to using MRI in the study of human peripheral atherosclerosis. Recent advances in sequence design, imaging speed, and surface coils have made possible rapid, high-resolution in vivo imaging of atherosclerosis.²⁷ Furthermore, its sensitivity to different chemical components of tissue can be exploited to noninvasively characterize plaque composition.^{28,29} Enthusiasm for the MR approach has already led to several studies evaluating atherosclerotic plaque progression in humans,^{30–33} but few have evaluated the use of MRI in measuring plaque in the peripheral circulation among PAD. In the most rigorously performed study to date, 7 patients with peripheral arterial occlusive disease were examined by both high-resolution MR imaging and IVUS.³⁴ The MR approach consisted of a 3-dimensional time-of-flight sequence used to measure vessel wall parameters with an in-plane resolution of $0.78 \times 0.49 \text{ mm}^2$ and slice thickness of 2 mm. High-resolution MR using this bright blood technique overestimated plaque area in the peripheral vessels compared with IVUS measurements. Moreover, posterior echo shadowing precluded reliable IVUS analysis in nearly half of the segments assessed, also highlighting the limitations of IVUS in PAD.

There are several potential advantages of imaging plaque volume in the SFA. The lack of SFA movement can significantly reduce artifacts compared with imaging in other vascular beds such as the carotids, coronaries, and aorta, which can be limited by swallowing or respiratory

motion. The relative proximity of the arteries in the leg to the body surface allows the use of high-resolution surface coils such as the one custom-built for this protocol. Another advantage is the sheer volume of atheroma that can be measured in the SFA, $7.27 \pm 3.73 \text{ cm}^3$ in this study, at least 2 orders of magnitude greater than volumes reported in either the carotid or coronary circulations. The large volume of plaque that can be quantified and excellent reproducibility of the measurements would reduce sample sizes needed to adequately power clinical studies using atherosclerotic plaque progression as a surrogate end point.

When estimating the potential impact of this technique on sample size estimates for clinical studies, one must consider the other noninvasive strategies currently being used. Using carotid intimal medial thickness measurements as a surrogate efficacy end point, Bots et al³⁵ reported that 468 patients would be required to detect a treatment effect of 30% (power, 0.80). Three-dimensional ultrasound of plaque in the carotids would require fewer patients to demonstrate efficacy, yet a recent study reported that 203 patients would still be needed to detect a 10% change in plaque volume (power, 0.90) measured during a 3-month period.³⁶ Measuring the progression of coronary calcium with cardiac computed tomography has been proposed as yet another noninvasive alternative to evaluating disease progression, yet sample sizes required for these studies remain large, and there is the strong suggestion that calcification represents a fundamentally distinct biological process that may correlate less with clinical events than plaque volume progression.^{37,38} Sample size estimates for our noninvasive approach are similar to that reported using coronary IVUS.²⁵

First-Pass Contrast-Enhanced Calf Muscle Perfusion at Peak Exercise

Using a dual-contrast technique, we found that perfusion differences were greatest between PAD and normals who achieve their maximal workload potential (NI_{max}). However, tissue perfusion remains a discriminating parameter even when workload is similar between PAD and normals. An additional advantage of this strategy is that it is semiquantitative and does not require extensive postprocessing.

A variety of noninvasive techniques have been used for the direct assessment of human skeletal muscle blood flow, including Doppler ultrasound^{39–41} scintigraphy,^{42,43} and plethysmography.^{44–46} The most widely accepted clinical standard for noninvasively measuring limb perfusion is venous occlusion strain gauge plethysmography. Aside from its inability to provide spatial localization of flow, plethysmography may underestimate perfusion and can be sensitive to changes in both body position and temperature.⁹ The temporally and spatially resolved data acquired with MR make it ideal for the study of lower limb perfusion. Consequently, a number of MR-based techniques have recently been evaluated in the assessment of lower limb blood flow.^{47–51} In each of these MR studies, predominantly healthy volunteers were evaluated during reactive hyperemia, and test characteristics were not assessed under low-flow conditions like that anticipated in patients with significant arterial insufficiency. Of the published techniques, those using arterial spin labeling^{47,52} and gadolinium first-pass enhancement seem most promising.^{51,53}

We modified a GRE pulse sequence to accommodate the simultaneous acquisition of muscle perfusion and the AIF. This approach was used to maximize sensitivity to contrast-enhancement in the muscle while avoiding saturation of the AIF signal. An MR-compatible, custom-built, 2-pedal plantar-flexion ergometer enabled patients to exercise while in the scanner environment, with real-time measures of work performed.

Recently, Thompson et al⁵³ introduced a somewhat different method for first-pass contrast-enhanced MRI in PAD. The technique relies on administration of contrast immediately after cuff inflation, both producing ischemia and allowing contrast to equilibrate in the arterial blood pool while excluding it from the lower limb. With cuff release, a true step-input of contrast

was produced that coincided with hyperemic blood flow. There are 3 main differences between the present approach and that of Thompson et al⁵³: (1) the use of exercise versus use of thigh occlusion to increase tissue blood flow, (2) the use of IR versus SR to generate T1-contrast, and (3) the use of tissue upslope normalized by AIF upslope as a PI versus use of a 2-compartment model for estimating the unidirectional influx constant and the contrast agent distribution volume with a cuff-generated step input.

In regard to the first difference, exercise has been shown to produce higher peak flows and more accurately reproduce physiological stress conditions⁵⁴ compared with postischemic reactive hyperemia. Furthermore, the pattern of limb perfusion at peak exercise reflects active muscle recruitment, rather than differences in fiber content and capillary density that govern regional flow in reactive hyperemia models.^{53,55} However, thigh occlusion may have the advantage of better reproducibility. For the second difference, IR would be expected to generate greater T1 contrast, although the pulse sequence repetition time may need to be longer. For the third difference, further studies are required to determine whether the normalized upslope or fitted model parameters are more sensitive and specific markers of PAD. For either case, an arterial input or a step function input can be used. For the thigh occlusion method, the availability of a step function input does simplify the analysis. However, if nonocclusion methods such as exercise or pharmacological vasodilation are used, then the AIF should be used to account for bolus dispersion and delay.

PCr Recovery Kinetics After Exercise

We found that PCr recovery time constant is prolonged in patients with symptomatic PAD when compared with normal individuals despite shorter exercise times and similar postexercise pH with an area under the ROC curve of 0.925. In addition, PCr recovery time is reproducible and may represent a parameter with which PAD patients can be both identified and followed, and this measure may identify PAD patients at higher risk of death and/or clinical deterioration requiring intervention.

Noninvasive modalities used for the diagnosis and study of PAD have a number of recognized limitations, including inaccuracy among patients with aortoiliac disease, diabetes mellitus, and collateralized territories.^{8,56} Heavily calcified arteries alone account for normal ABI values in as many as 1 in 20 PAD patients.⁵⁷ Both x-ray angiography and MRA are limited to visualizing the vascular lumen. With these techniques, macrovascular abnormalities serve as a surrogate marker of tissue ischemia, largely ignoring adaptations in cellular metabolism and within the microvasculature that develop during the evolution of vascular insufficiency and influence end-organ response.^{11,13}

Experimental evidence from histopathologic and clinical studies suggests that skeletal muscle is not a passive bystander during development of peripheral vascular obstruction. Cellular alterations in PAD are comparable to those in subjects with mitochondrial myopathies and include altered mitochondrial enzyme expression, accumulation of the intermediates of oxidative metabolism, and mitochondrial DNA damage.^{11,12} Both biochemical and ultrastructural changes may in part explain why revascularization does not normalize measures of clinical performance in many PAD patients.⁵⁸ Although some metabolic changes may be maladaptive, others, such as an increase in mitochondrial density and more efficient glycolytic metabolism,⁵⁹ can lead to improvement in symptoms and exercise capacity in the absence of revascularization and may underlie the benefit of physical training in PAD.⁶⁰

The major role of PCr in muscle metabolism is as a transport molecule and reservoir of high-energy phosphate bonds, the sole currency of cellular energetics.⁶¹ Phosphocreatine regeneration occurs exclusively within the mitochondria, and because this depends entirely on the cell's capacity for oxidative phosphorylation and oxygen supply,⁶² it represents an ideal

parameter for detecting metabolic evidence of tissue ischemia. Relationships between peak oxygen uptake, maximal oxygen consumption, and PCr recovery kinetics have been well documented.^{63,64}

Phosphocreatine recovery time clearly distinguished symptomatic PAD patients from normal controls. These findings are in accordance with other studies that have demonstrated delayed regeneration of PCr as a reliable marker of arterial obstruction.^{13,58,65,66} When compared with normal subjects in our study, there was an approximate 3-fold increase in the recovery time constant among patients. The area under the fitted curve of the ROC analysis is 0.925, demonstrating that PCr recovery kinetics can be used to discriminate mild to moderate symptomatic PAD patients from normals.

The median pH at end exercise was 7.02 in PAD and 6.95 in controls ($P = 0.19$). Hence, prolongation in PCr recovery was not the result of greater acidosis at end exercise among PAD. This is important because tissue pH can influence PCr recovery kinetics. Not only is creatine kinase a pH-dependent enzyme,⁶² but as much as 40% of cellular ATP may be consumed in ion pumping reactions during significant acidosis.⁶⁴ With this ATP no longer available to facilitate PCr regeneration, recovery time is prolonged. Furthermore, PCr/ATP ratios were similar at both rest and peak exercise, suggesting that differences in PCr recovery times were not the consequence of greater energy debt among PAD. Moreover, significant left ventricular systolic dysfunction was not responsible for prolonged PCr recovery times.

During clinical follow-up, revascularization was performed on 6 patients, and 1 individual died. Although only a trend, our data suggest longer PCr recovery time constants among mild to moderate PAD at greater risk for death, subsequent limb revascularization, or amputation ($P = 0.057$). This was most apparent among patients in the highest quartile of PCr recovery kinetics ($RR = 3.11$). No such trend was noted between these relevant clinical events and the resting ABI. This lack of correlation between the ABI and clinical events may be due to inherent inadequacies in the test itself because it evaluates primarily macrovascular hemodynamics, rather than true tissue perfusion. Although an excellent tool for identifying PAD with a sensitivity and specificity of 90% and 98%, respectively,⁴ the ABI frequently fails to predict functional capacity, quality of life, symptoms, and clinical deterioration among the subgroup of patients with mild to moderate disease.^{57,67–69} A diagnostic tool capable of predicting clinical deterioration or death among patients with mild to moderate PAD would be invaluable, and thus, the relationship between PCr recovery kinetics and clinical outcomes warrants further study.

Limitations of These Techniques

SFA Plaque Volume Measurements: For black-blood plaque imaging, the ability to accurately define the luminal border of the vessel was predicated on the generation of black blood. Whether using IR or flow saturation bands, as done in our studies, black-blood contrast requires adequate inflow from outside of the selected slice. A certain percentage of patients even with mild to moderate PAD present with complete occlusion of the SFA, making measurement of plaque volume in that artery impossible. The reliability and reproducibility of this imaging strategy may suffer further when performed in patients with more advanced vascular disease and significantly reduced flow with potentially higher rates of SFA occlusion. Because we used a small-element surface coil, vessel image quality suffered in more obese patients. A larger coil array and longer scan times with more signal averages would improve image quality in larger patients. We also found that image quality diminished in the distal one fourth of the SFA in some patients, typically as the vessel coursed deeper into the tissue and posteriorly into the popliteal fossa. This can be problematic because the adductor canal region of the SFA has a known predilection for atherosclerosis and is a common site of disease progression.^{70,71} In the coronary vasculature, even modest changes in plaque progression may

be associated with large differences in vascular outcomes.^{19,20,72} Whether this relationship between plaque progression and vascular events holds true in the periphery remains undetermined.

Sample size estimates are based on plaque volumes measured in patients with established PAD. In clinical studies evaluating therapeutic efficacy among patients with subclinical atherosclerosis, larger total sample sizes may be required using our approach because of smaller total plaque burden in this population. Although required sample sizes may be reduced with measurement of plaque volume progression in the SFA, total imaging costs may be similar to that of other techniques because of the increased expense of MRI. However, these imaging costs would be small compared with the potential savings realized by having a substantially smaller patient cohort to enroll, image, analyze, and follow.

First-Pass Contrast-Enhanced Calf Muscle Perfusion at Peak Exercise: A limitation of this methodology was lack of validation with a separate technique capable of making absolute measures of blood flow. The upslope ratio (PI) used to compare subjects in this protocol is semiquantitative, providing only a partial description of the perfusion system. Nevertheless, this approach accounts for variations in the AIF that can be a source of error. In studies of myocardial first-pass perfusion, the upslope ratio tends to lead to a systematic underestimation of blood flow at higher flow rates.⁷³ This phenomenon could have influenced our findings in the lower limbs and underestimated true differences in perfusion between groups. However, in the study by Christian et al,⁷³ underestimation of flow was most problematic at peak flow rates greater than 200 to 250 mL min⁻¹ 100 g⁻¹ tissue, peak values at the upper limit of that typically reported in the limbs of normal controls^{52–54,74} and well less than that described in PAD.⁴³

The NI_{max} group was younger than the PAD group. Although age-related differences between NI_{max} and PAD can exaggerate differences in perfusion between these 2 groups, studies have demonstrated that active muscle perfusion remains intact in healthy older individuals.^{75,76} In all subjects in this protocol, we observed a heterogeneous pattern of limb perfusion after exercise consistent with previous investigators.^{52,53,77} However, the pattern of perfusion heterogeneity we observed differed among some individuals, suggesting muscle groups being used to perform exercise on the ergometer were inconsistent, and this could have skewed our comparisons. Patients with mild to moderate PAD were studied. Patients with more severe forms of PAD with critical limb ischemia and occlusion of the SFA and other vessels may not be candidates for this approach because measuring the arterial input would not be feasible.

Inflow effects can result in distortions of the measured AIF curves. To minimize these effects, we applied flow-compensated gradient waveforms and used an echo train length of 1, whereas prior studies⁵¹ used echo train length of 2, which amplified flow artifact, and no flow compensation. In addition, in data processing, we estimated the slope of the AIF, which is less sensitive to AIF oscillations than the deconvolution analysis used previously.⁵¹ Pulsatile arterial flow did lead to ghosting artifact and small oscillations in the measured AIFs in our studies.

Test-retest reliability studies were not performed for these measures. Variability between sites regarding the details of the pulse sequence, receive coil, exercise protocol, and patient characteristics may limit reproducibility of these methods to other centers. If surface coils are used for signal reception, coil profile effects will need to be accounted for before calculating TIC slopes.

PCr Recovery Kinetics After Exercise: To optimize temporal resolution and retain adequate signal to measure both pH and PCr/ATP ratios, a surface-coil acquisition localized to the

midcalf was used with the understanding that both exercising and nonexercising muscle groups would be incorporated into the signal, which might result in the underestimation of PCr depletion in the most stressed muscle groups.⁴⁸ With the knowledge that PCr recovery time is the most relevant parameter and resolution of Pi and ATP peaks less important, echo-planar spectroscopic imaging can be used to interrogate individual muscle groups with adequate temporal resolution to assess PCr recovery kinetics.⁷⁸

Baseline age and sex differences between controls and patients might account for differences in PCr recovery time. However, others have demonstrated a lack of correlation between age and PCr recovery time,^{79,80} and no age-related differences were noted within the present cohort. Although age-related changes in muscle mass, fiber type, and even oxidative capacity have been reported,⁸¹ a number of studies have demonstrated that active muscle perfusion remains intact among healthy older individuals.^{76,82} Hence, baseline differences in age may only be relevant under conditions in which blood flow is minimally perturbed (submaximal exercise) and mitochondrial function becomes the rate-limiting step in PCr regeneration.⁵⁸

Total work and the rate and degree of plantarflexion were not controlled in our protocol. Prolongation of PCr regeneration does not pinpoint the level of vascular obstruction in PAD patients and has been described in other disease states where substrate supply may be limited or a primary metabolic defect exists.^{83,84} For example, in heart-failure patients, delayed PCr recovery has been attributed to decreased mitochondrial volume and impaired oxidative metabolism, rather than central hemodynamic factors.⁸³ Many of the PAD patients have coronary artery disease. Myocardial ischemia and/or LV dysfunction that may have developed during the exercise protocol can have confounded our results. However, no patients experienced chest pain or other clinical events during the exercise protocol. Furthermore, mean resting ejection fraction was preserved among patients in whom it was measured clinically before study (ejection fraction, $56\% \pm 12\%$). With respect to clinical events, the sample size is too small to draw any definitive conclusions regarding the prognostic power of PCr recovery kinetics.

Future Directions—Serial measurements of SFA plaque volume can be used to better understand the natural history of PAD and characterize plaque volume and distribution in different PAD patient subsets, including those who are asymptomatic, have atypical symptoms, or are diabetic. Identification of plaque characteristics that can be used to recognize PAD at highest risk for adverse clinical outcomes in the lower extremities would be of significant clinical import. Furthermore, serial noninvasive measurement of plaque volume with MRI can allow for the study and validation of both established and novel therapies for PAD, including behavioral, pharmacological, genetic, stem cell, and percutaneous interventions. Correlation of plaque progression in the peripheral circulation with vascular events in the coronary, cerebral, and peripheral circulation is also warranted.

Improving blood flow at a tissue level is a major therapeutic goal in the treatment of patients with PAD. Although several therapies proven beneficial in the treatment of symptomatic PAD ostensibly work through enhancement of skeletal muscle perfusion,^{85–87} the true mechanism of action of these therapies has remained elusive because techniques capable of reliably measuring tissue blood flow have been lacking. Correlation of clinical efficacy with changes in tissue blood flow at peak exercise will help us to better understand our current treatment approach and will be critical in the development and validation of new therapeutic strategies for PAD patients. Because the skeletal muscle does not remain a passive bystander during the development of PAD, association of tissue perfusion with noninvasive measures of skeletal muscle metabolism, and cellular energetics will also be important in understanding patient symptoms, performance measures, and clinical outcomes.¹⁶

^{31}P MRS has a number of potential applications in the PAD population. The relationship between PCr recovery kinetics and clinical events involving the lower limbs deserves further exploration in a larger cohort of patients. Investigation of asymptomatic patients and those who experience atypical symptoms may provide insight into the biochemical characteristics that distinguish these groups. Whether differences in PCr recovery time result entirely from changes in tissue blood flow, alterations in skeletal muscle at a cellular level, or a combination of both, deserves further investigation.

All of these techniques taken together can serve as a platform to study the surgical, percutaneous, and medical interventions currently used as well as the investigational therapies of the future.

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FIGURE 1.

Representative sequential images (upper left to lower right) from the femoral artery of a subject with mild to moderate PAD with both the luminal and adventitial border clearly delineated. Note the slice-to-slice variation in plaque morphology. Reprinted with permission from Isbell et al. *J Cardiovasc Magn Reson.* 2007;9:71–76.¹⁴

FIGURE 2.

The scout image (left) shows the location of both the upper SR slice, from which the AIF is derived, and the lower IR slice, which yields the TF. Representative images from each slice are shown (right upper and lower panels) during contrast infusion in a normal subject at peak exercise with regions of interest delineated. Note the heterogenous pattern of enhancement (right lower panel) in the muscle groups of the lower leg. In addition, note the differences in image contrast between the upper SR slice and lower IR slice, with nulling of nonenhanced muscle in the IR slice providing greater sensitivity to low concentrations of contrast agent. Representative TICs (bottom) of the AIF and TF are shown. TF indicates tissue function. Reprinted with permission from Isbell et al. *J Magn Reson Imaging*. 2007;25:1013–1020.¹⁵

FIGURE 3.

Workload (in joules) achieved between the 3 experimental groups. The central line represents the median, whereas the outer borders of the box represent the 25th and 75th percentiles of the data. NL_{max} achieved a higher workload than both PAD and NL_{low}. Reprinted with permission from Isbell et al. *J Magn Reson Imaging*. 2007;25:1013–1020.¹⁵

FIGURE 4.

Perfusion index compared between the 3 experimental groups. The central line represents the median, whereas the outer borders of the box represent the 25th and 75th percentiles of the data. PI discriminates PAD from normals, irrespective of the workload achieved. There is a strong trend toward increased PI in NL_{max} compared with NL_{low}. Reprinted with permission from Isbell et al. *J Magn Reson Imaging*. 2007;25:1013–1020.¹⁵

FIGURE 5.

Representative PCr recovery plots in a control (A) and PAD (B) subject. Note the steeper recovery curve in the control subject. The PCr recovery time constant was 34.4 seconds in control and 121.1 seconds in the PAD patient. Reprinted with permission from Isbell et al. *J Am Coll Cardiol.* 2006;47:2289–2297.¹⁶

FIGURE 6.

In this graph of PCr recovery time constants, the boxes extend from the 25th to the 75th percentile, and the horizontal lines depict the medians. The median PCr recovery time constant is longer in PAD patients. Reprinted with permission from Isbell et al. *J Am Coll Cardiol.* 2006;47:2289–2297.¹⁶

TABLE 1
Sample Size Estimates for Clinical Trials of Changes in APV Using MRI in the Superficial Femoral Artery

Mean Δ APV (%)	N (power, 0.80)	N (power, 0.90)
1	107	142
2	27	36
3	12	16
5	5	6