Published in final edited form as:

Am Heart J. 2017 January ; 183: 24–34. doi:10.1016/j.ahj.2016.09.013.

Baseline Assessment and Comparison of Arterial Anatomy, Hyperemic Flow and Skeletal Muscle Perfusion in Peripheral Artery Disease: The CCTRN PACE Study

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Abstract

Background—Peripheral artery disease (PAD) is important to public health as a major contributor to cardiovascular morbidity and mortality. Recent developments in MRI techniques permit improved assessment of PAD anatomy and physiology, and may serve as surrogate endpoints after pro-angiogenic therapies.

Methods—The “Patients with Intermittent Claudication Injected with ALDH Bright Cells” (PACE) study is a randomized, double-blind, placebo-controlled clinical trial designed to assess the physiologic impact and potential clinical efficacy of autologous bone marrow-derived ALDH^{br} stem cells. The primary MRI endpoints of the study are; 1) total collateral count, 2) calf muscle plasma volume (a measure of capillary perfusion) by dynamic contrast-enhanced MRI, and 3) peak hyperemic popliteal flow by phase contrast MRI.

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Relationship to industry: None

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Results—The inter- and intra-reader and test-retest results demonstrated good-to-excellent reproducibility (ICC range: 0.61–0.98) for all MR measures. The PAD participants (n=82) had lower capillary perfusion measured by calf muscle plasma volume (3.8 vs 5.6%), and peak hyperemic popliteal flow (4.1 vs 13.5 ml/s) as compared to the normal participants (n=16), with a significant level of collateralization.

Conclusions—Reproducibility of the MRI primary endpoints in PACE was very good to excellent. The PAD participants exhibited decreased calf muscle capillary perfusion as well as arterial flow reserve when compared to normal participants. The MRI tools utilized in PACE may advance PAD science by enabling accurate measurement of PAD microvascular anatomy and perfusion before and after stem cell or other PAD therapies.

INTRODUCTION

The prevalence of peripheral artery disease (PAD) with its attendant high risk of heart attack, stroke, amputation, and death identifies it as a major cardiovascular public health priority.(1) PAD is characterized by the presence of a stenosis or occlusion of infrarenal arteries. The primary ischemic leg symptom for individuals with PAD is claudication which represents the exertional muscle fatigue induced by a supply-demand mismatch provoked by walking. While many effective claudication treatments exist (supervised exercise, claudication pharmacotherapy, endovascular and open surgical revascularization), no current therapy provides an ideally effective approach for all patients. Furthermore, the mechanisms which underlie clinical efficacy of proven claudication treatments remain uncertain.

Stem cell therapies have been proposed as a potential treatment option due to their presumed angiogenicity, associated with improvements in tissue oxygenation and ischemic muscle perfusion.(2, 3) However, the question of whether the stimulation of collateral blood vessel growth or modification of existing arteries remains in the causal pathway remains unclear.

Magnetic resonance imaging (MRI) can assess arterial anatomy using contrast-enhanced magnetic resonance angiography (MRA) In addition, MRI has the ability to measure muscle perfusion and vascular reactivity.(4) Inasmuch as MRI uniquely provides both anatomic and physiologic data, the Cardiovascular Cell Therapy Research Network (CCTRN) chose MRI imaging endpoints for three of its four primary endpoints in the PACE clinical trial. Those endpoints include the change from baseline to six months in 1) total collateral count (thigh and calf) from MRA, 2) calf muscle plasma volume (capillary perfusion) as measured by dynamic contrast-enhanced MRI (DCE-MRI), and 3) peak hyperemic popliteal flow by phase contrast MRI. This paper describes the method of their MRI -based determination, and assesses the relationship between these MRI measurements in patients with and without PAD.

METHODS/DESIGN

The CCTRN “Patients with Intermittent Claudication Injected with ALDH Bright Cells” (PACE) is a randomized, double-blind, placebo-controlled clinical trial designed to assess the clinical safety and efficacy of autologous bone marrow derived ALDH^{br} cells delivered by direct intramuscular injections in patients with PAD and limiting claudication symptoms.

Funding for PACE was provided to the CCTRN by the National Heart, Lung, and Blood Institute (NHLBI) at the National Institutes of Health. The authors are solely responsible for the design and conduct of this trial, all trial analyses, the drafting and editing of the paper and its final contents. The detailed study design, including the inclusion and exclusion criteria, sample size, and outcomes have been previously published.⁽⁵⁾ Primary study outcomes include both clinical endpoint (change in peak walking time) and physiologic MRI-derived measurements of leg collateral arterial anatomy, arterial blood flow, and capillary perfusion at six months compared to baseline.⁽⁵⁾

MRI Protocol Design and Analysis

As shown in protocol operations flow-chart (Figure 1), patient preparation included intravenous access for MRI contrast agent injection, placement of cuffs for inducing reactive hyperemia, and positioning of the MRI coils for signal reception. Gadopentate dimeglumine (MagnevistTM) was chosen as the contrast agent due to its availability at all study sites.

All image post-processing and data analysis were performed at the core lab (Johns Hopkins University, Baltimore, MD). All site personnel were trained in implementation of the imaging protocol and proper handling of clinical trial data. The technologists were required to undergo on-site training and participate in an on-line webinar prior to site certification. For each study, a detailed check for image quality and usability was performed and appropriate feedback conveyed to the technologists. Table 1 provides a list of the scanner types, magnet strength, and coil availability at each site.

Calf muscle reactive hyperemia—A customized MRI-compatible thigh blood pressure cuff (Magmedix Inc, MA, USA) was used for thigh arterial occlusion of the index leg, by cuff inflation to the “occlusion” pressure for 5 minutes, to induce calf skeletal muscle ischemia. The lowest thigh cuff pressure, causing complete arterial occlusion demonstrated by abolition of pedal artery flow seen on a Doppler probe, was identified as the occlusion pressure to minimize subject discomfort during the MR imaging.⁽⁶⁾

Localizers—MRI imaging started with the acquisition of multi-planar scout images covering the upper and lower legs and was followed by 2D time-of-flight imaging to obtain low-resolution images of the underlying arterial anatomy.

Dynamic contrast-enhanced MRI—The thickest portion of the calf was identified from the prior acquired sagittal scout and 3D reconstructed time-of-flight images. Tissue spin-lattice relaxation time (T1) maps of 8 transverse slices centered at the thickest portion of the calf was calculated using a multiple flip-angle method with same positioning and imaging parameters for the DCE-MRI sequence but with varying flip angles of 5°, 8°, 12° and 15°. (7) The DCE-MRI data acquisition of the calf was performed with a spoiled fast gradient echo sequence and a flip angle of 15°: slice thickness of 5 mm, slice gap = 1 mm, in-plane acquired spatial resolution of 1 mm × 1mm, TR/TE ~ 2.5 ms/1 ms, and parallel imaging factor of 2. This yielded a temporal resolution of approximately 3–4 s per dynamic, and the continuous acquisition of 100 dynamics results in a scan time of 5–6 minutes. The cuff was inflated to the occlusion pressure, and then, a 0.05 mmol/kg dose of gadolinium (followed

by 1 mL of saline flush) was administered at the rate of 1.5 cc/s. The contrast agent pooled in the arteries above the inflated cuff for 5 minutes as hyperemia was induced in the calf muscle. The DCE MRI sequence was started 20 seconds before the cuff deflation and continued to run as the cuff rapidly deflated. The DCE-MRI images were analyzed using the DCE Tool plugin (http://kyungs.bol.ucla.edu/software/DCE_tool/DCE_tool.html) in Osirix (Figure 2).

An affine registration algorithm with conjugate gradient minimization of the mutual information between the reference (flip-angle 15° images from multiple flip-angle imaging) and moving images (DCE images) was used for registering the images(8) in MATLAB (Mathworks, Natick, MA). After estimation of the T1 map, the observed signal intensities on the DCE images were converted to contrast agent concentrations. An arterial input function (AIF) was drawn corresponding to the most prominent artery visually identified by the user. Failing the identification of a prominent artery, a population-averaged AIF was used.(9) The user manually placed regions of interest in the anterior tibial, gastrocnemius and soleus muscles resulting in three volumes of 1 cm³ each. Quantitative analysis using a modified Tofts' model yielded the transfer constant from the blood plasma to the extracellular extravascular space (Ktrans) and the whole blood plasma volume per unit of tissue (Vp).(10) Change over 6 months in calf muscle plasma volume (a measure of capillary perfusion) was a primary endpoint of the PACE trial.

Phase-contrast MRI—2D phase-contrast MRI (PC-MRI) of the popliteal artery with electrocardiographic gating was performed with the following acquisition parameters: spatial resolution 1.2 mm × 1.2 mm, slice thickness 5 mm, TR/TE ~ 10 ms/2.2 ms, flip angle 25°, parallel imaging factor 2, velocity encoding of 100 cm/s (increased by 50 cm/s if aliasing observed) and time of acquisition per scan 30–40 s. The prescribed imaging plane was perpendicular to the long-axis of the popliteal (Figure 3).

The protocol acquired 3 PC-MRI dynamics before cuff compression to estimate baseline blood flow as well as its short-term variability. PC-MRI acquisitions were then performed consecutively for 10 minutes from the start of cuff deflation. Flow curves were generated using QFlow (v5.2, Medis, Leiden, Netherlands) following a methodology described previously.(11) From the generated flow curve, the peak instantaneous cardiac blood flow (characterized for each dynamic) within that cardiac cycle was noted. Measured parameters included resting flow (average peak flow at rest over 3 acquisitions), peak hyperemic popliteal flow (peak flow after cuff deflation as observed over 10 minutes), time-to-peak hyperemic flow (from the point of cuff deflation) and arterial flow reserve (peak hyperemic flow – resting flow). Change over 6 months in peak hyperemic popliteal flow is a primary endpoint of the PACE trial.

Contrast-enhanced MRA—Contrast-enhanced MRA of the thigh and the calf were performed separately with an overlap in the volume of interest to ensure complete coverage of the lower extremity arteries from the bifurcation of the common femoral artery to the pedal arteries.

Thigh MRA: A 3D spoiled gradient echo sequence was used to obtain one pre-contrast and 3 post-contrast MRA scans of the thigh using the following parameters: $1.3\text{mm} \times 1\text{mm} \times 1.3\text{mm}$ spatial resolution, TR/TE $\sim 2.5/1\text{ ms}$, a parallel imaging factor 2 and a time of acquisition per sequence $\sim 15\text{--}22\text{ s}$. The volume of interest in the thigh spanned the bifurcation of the femoral artery to the trifurcation of the popliteal artery (Figure 5).

After a 0.1 mmol/kg dose of gadolinium (followed by 10 mL of saline) was injected at the rate of 1.5 cc/s , fluoroscopic triggering was used to initiate the post-contrast scans.

Calf MRA: Time-resolved magnetic resonance angiography (t-MRA) via an ultrafast high resolution 3D dynamic scan was used to assess calf blood flow kinetics with acquisition parameters: isotropic spatial resolution $\sim 1\text{mm} \times 1\text{mm} \times 1\text{mm}$, 25° flip angle, TR/TE $\sim 3\text{ ms}/1\text{ ms}$, a parallel imaging factor 2, and vendor specific under-sampling methods (TWIST in Siemens, TRICKS in GE and Keyhole/4D TRAK in Philips) to obtain a temporal resolution of $8\text{--}12\text{ s}$ per dynamic. The volume of interest in the calf spanned the region from the trifurcation of the popliteal artery to the pedal arteries (Figure 4).

A single pre-contrast acquisition was performed, and then a 0.05 mmol/kg dose of gadolinium was administered at a rate of 1.5 cc/s followed by 10 ml of saline flush. Simultaneous to contrast injection, the post-contrast MRA acquisitions were started and continued for 2 minutes.

MRA Image Analysis: The degree of arterial vascularization was assessed manually by quantifying the average number of conduit, collateral and terminal branch arteries crossing 5 transverse planes in the thigh (a reference plane at the highest grade stenosis, three proximal and one distal to it) 5 cm apart, using the dynamic scans that depicted the arteries with the highest contrast-to-noise ratio via visual inspection(4) (Figure 4). The extent of arterial collateralization at the thigh and calf was measured by quantifying the number of vessels that bypassed a stenosis and reconstituted the main conduit vessel (Figures 4, 5). In addition, the number and the degree of stenosis in the thigh and calf arteries were measured. At the thigh, the superficial femoral artery was divided along its length into three equal segments with the degree of stenosis measured and reported for the tightest lesion in each segment. At the calf, the tightest stenosis in each of the anterior tibial, the posterior tibial and the peroneal artery was measured and reported. All MRA images were reviewed on a dedicated workstation (AZE Technologies, Japan). The change over 6 months in the total collateral count measured at the thigh and at the calf is a primary endpoint of the PACE trial.

Population

82 participants with PAD were recruited across 9 sites over a period of 30 months (June 2013 to January 2016), with half assigned to a treatment group and half to a placebo group. All PAD participants had symptoms of intermittent claudication, a significant stenosis or occlusion of infrainguinal arteries and a resting ankle-brachial index less than 0.90 or resting toe-brachial index of less than 0.70. Subjects with critical limb ischemia or a contraindication to MRI were excluded. 16 participants from 8 clinical sites (1–3 studies per site) were evaluated for the PACE MRI reproducibility sub-study. All measures were analyzed by 2 experienced analysts blinded to each other's analysis for inter-reader

reproducibility assessment. These parameters were then measured a second time by one of the analysts blinded to the first interpretation and at least 2 weeks apart to assess intra-reader reproducibility.

Test-retest reproducibility was performed at 1.5 Tesla (T) (Siemens Avanto) and 3T (Siemens Trio) scanners in 16 males and females (8 at 1.5T and 8 at 3T) at Johns Hopkins University, Baltimore, MD without a prior diagnosis of PAD (based on self-reported questionnaires) recruited from the community to serve as normal control participants for comparison of MRI measures. The entire protocol was performed twice – at baseline and a repeat scan one month later (average: 32 ± 5 days). All parameters were measured by one experienced reader who was blinded to the baseline read when assessing the repeat scan.

Both protocols were approved by the Institutional Review Boards of participating organizations and all participants signed informed consent.

Statistical Analysis

The interclass correlation coefficient (ICC) was used to measure the agreement between the reads for continuous variables and the Kappa concordance test was used for categorical variables. A two-sample t-test was used to determine the significance of differences in the functional parameters between PAD and normal participants for continuous variables, and Fisher's exact test for discrete variables. Additional pairwise comparisons were performed after age, gender and scanner field strength adjustment using the general linear model. $P < 0.05$ was considered statistically significant. Statistical analysis was performed with commercially available statistical software (STATA, College Station, TX).

RESULTS

Reproducibility Assessment

One of the primary endpoints of PACE – capillary perfusion measured by calf muscle plasma volume (V_p) demonstrated good-to-excellent inter- and intra-reader reproducibility (ICC range: 0.78, 0.90). PC-MRI measures also demonstrated excellent inter- and intra-reader reproducibility, including the primary endpoint - peak hyperemic popliteal flow (ICC: 0.95) and other measures of PC-MRI assessed in the index leg (i.e., resting flow, arterial flow reserve, and time-to-peak flow after cuff deflation) (ICC range: 0.71–0.98). Table 2 shows the mean values and ICCs for each parameter.

The third primary MRI endpoint, the total collateral count at the thigh and calf also demonstrated high reproducibility (Table 3).

The measurements of two of the PACE MRI endpoints, capillary perfusion measured by calf muscle plasma volume and peak hyperemic popliteal flow, as well as other PACE measures (ktrans, resting flow, and arterial flow reserve) using DCE-MRI and PC-MRI demonstrated good-to-excellent test-retest reproducibility with a small ICC (range 0.73–0.94) as shown in Table 4.

Neither arterial stenoses nor occlusions were observed in the normal volunteers in the thigh or calf muscles. Test-retest reproducibility was moderate-to-good for the quantification of number of arteries per plane.

PAD vs. normal participants

The PAD patients in our study were significantly older than the normal group (Table 5). In 4 of the 82 PAD participants, an AIF was not recognized and hence a population-averaged AIF was used. Capillary perfusion measured by calf muscle plasma volume (Vp) and ktrans were significantly higher in the normal group as compared to the patients (Figure 7).

The differences remained significant after adjustment for age, gender and scanner magnetic field strength. The resting flow, peak hyperemic popliteal flow and arterial flow reserve were higher, but the time-to-peak flow after cuff deflation was lower, for normal participants compared to PAD participants. In 5 of the PAD participants, there was complete occlusion of the popliteal artery and hence all flow measures were characterized as 0 ml/s and time-to-peak flow was not calculated. The number of arteries per plane at the point of occlusion in the thigh for PAD patients was not significantly different from the number of arteries per plane characterized at the thigh level in the normal participants. While no collateralization was observed in the normal participants, there was significant collateralization observed in the PAD participants (thigh = 3.6 ± 3.3 , calf = 1.1 ± 2), total = 4.7 ± 4.0 (mean $\pm$ SD)).

DISCUSSION

The PACE clinical trial was designed to evaluate the utility of a series of potentially important but novel PAD-specific MRI endpoints. The primary MRI endpoints of the study were chosen to assess change over 6 months in the total collateral count (calf and thigh), capillary perfusion measured by calf muscle plasma volume and peak hyperemic popliteal flow. Theoretically, these calf muscle MRI measurements may provide important new objective PAD anatomic and physiologic data that could define the potential efficacy of this cell-based investigational intervention. The results of the preliminary work described here demonstrated good to excellent reproducibility for the MRI endpoints chosen for PACE. In addition, capillary perfusion measured by calf muscle plasma volume and peak hyperemic popliteal flow were lower in the PACE participants at baseline than in normals, while the PACE participants showed a high degree of collateralization. The ability of MRI measurements to provide objective evidence of new cell-mediated collateral growth fulfilled a key PACE trial objective.

PAD clinical trials designed to collect baseline and post-treatment MRI data confront key challenges associated with the implementation of complex imaging protocol in a multi-center setting. We identified several challenges in implementing a uniform imaging protocol across the participating sites - (1) varying field strengths (1.5-T and 3.0-T) have differential effects on the observed signal-to-noise ratios and susceptibility to artifacts; (2) variations in gradient performance and peak transmit radiofrequency amplitude across sites may limit the ability to adjust certain imaging parameters; (3) vendors may vary on under-sampling strategies and parallel imaging techniques; and (4) centers may use different MRI contrast agents leading to different contrast agent relaxivities, binding mechanisms, as well as

biological clearance times affecting imaging.(12) The present study created site qualification standards, continuous quality assurance efforts, and a standardized image acquisition process to ensure reliability and comparability, and demonstrated that all of the endpoints had moderate to excellent intra- and inter-reader reproducibility as well as moderate to high test-retest reproducibility.

MRI also has been used to measure angiogenesis during evaluation of tumors using T1-weighted DCE-MRI perfusion imaging.(15, 16) DCE-MRI is a technique to quantify microcirculation to measure the rate of vascular reactivity and capillary perfusion.(17, 18) These measures showed the ability to discriminate between PAD and normal participants, especially in hyperemic conditions.(7, 12, 19–21) Indeed, similar to previous studies, we found that there was a significant difference between PAD and normal participants in both capillary perfusion measured by calf muscle plasma volume and the transfer constant also showing high reproducibility. Identification of a pre-defined plane for acquisition of the arterial input function located proximal to the imaging plane is complicated by the possibility of there being an occlusion or even a partial stenosis of the arterial wall changing flow characteristics. To minimize these effects and to make implementation of the protocol easier across sites, measurement of arterial input function was performed at the same slice. Arterial flow reserve might provide additional diagnostic and physiologic data as compared to measurements of resting leg blood flow alone, and have shown high reproducibility.(11, 22) Similar to previous demonstrations(11), we showed that both resting flow and peak hyperemic popliteal flow were lower in PAD participants compared to normal participants, indicative of decreased arterial flow reserve in the PAD population. The time-to-peak flow after cuff release (deflation), as demonstrated earlier, was higher in PAD participants, indicative of decreased maximal arteriolar vasodilation.

Study Limitations

This study evaluated the test-retest reproducibility in normal participants in order to decrease the risk of repeated administration of gadolinium in individuals with PAD. It is possible that these reproducibility data would be different in older and less healthy individuals with PAD. Similarly, the sample size of this reproducibility study is small, although it is comparable to other MRI reproducibility studies.(4, 20) Furthermore, nine sites were used in this clinical trial and achievement of comparable quality assurance may prove to be more challenging with a higher number of study sites in larger studies. Also, the normal participants in this study were younger than the PAD patients. We minimized this effect by adjustments for age and gender in our comparisons, however, this may not be ideal. Finally, MRI was used to evaluate leg PAD anatomy, popliteal artery blood flow, and calf perfusion; however, it remains possible that these variables do not represent the key mechanisms that underpin claudication physiology. Other potentially important measures, such as skeletal muscle innervation, conditioning, muscle cytochrome oxidative metabolism, and other factors are not measured by these techniques. No comparisons were made with computed tomography, ultrasound or invasive angiography, therefore extension to other imaging modalities may not be straightforward.

CONCLUSION

An advanced MRI protocol and associated analytic methodology, supported by a core laboratory, can provide reproducible, high fidelity data to accurately measure PAD native artery anatomy and disease severity, total collateral count, peak hyperemic popliteal flow, and capillary perfusion measured by calf muscle plasma volume. These methods were reproducible and differentiated between PAD and normal participants. These measurements in individuals with PAD at baseline and following stem cell administration, within a prospective, placebo-controlled, multi-center clinical trial (PACE) will evaluate the hypothesis that stem cell administration may benefit calf muscle perfusion and associated clinical PAD symptoms.

Acknowledgments

PACE funded by NIH network grants (NHLBI UM1HL087318). The views expressed in this paper by one of the authors (RFE) do not necessarily reflect those of the National Heart, Lung, and Blood Institute, the National Institutes of Health, or the US Department of Health and Human Services.

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Figure 1. Protocol flowchart including the gadolinium-dimeglumine contrast dose and reactive hyperemia cuff compression technique. Survey included localizers and 2D time-of-flight sequences as these images were used for planning and positioning of future data acquisition. T1 mapping used a multiple flip-angle method as an essential component of accurate perfusion measurement. DCE-MRI (dynamic contrast enhanced magnetic resonance imaging) was performed using fast gradient echo sequences to enable measurement of capillary perfusion by calf muscle plasma volume following cuff-induced hyperemia. PC-MRI (2D phase contrast MRI) measured the flow through the popliteal artery at both rest and hyperemic state to enable measurement of vascular reactivity. Contrast enhanced MRA (MR angiography) of the upper and lower legs were performed separately using time-resolved angiographic sequences to assess stenosis and collateral artery anatomy.

Figure 2.

An axial image of a transversal slice of the calf muscle from DCE-MRI and the corresponding enhancement graph for the symptomatic leg (right leg). The red region-of-interest indicates the pixels used to obtain the arterial-input-function and the other regions-of-interest indicates delineated skeletal muscle regions.

Figure 3.

2D Phase Contrast-MRI images (left) using the cuff paradigm before. The graph on the right shows the peak hyperemic popliteal flow measured in a normal volunteer (blue curve) and a patient with significant stenosis of the popliteal artery (red) up to 8 minutes after cuff release. The cuff was inflated to supra-systolic pressures for 5 minutes in both cases.

Figure 4.

Coronal maximum intensity projection (MIP) obtained after subtraction of pre-contrast images from post-contrast images for two consecutive acquisitions of MRA of the thighs showing complete stenosis and collateralization (arrows) in a 55 year-old male. Note 5 transverse planes (red lines) set 5 cm apart and deployed above and below the most significant stenosis in the symptomatic leg (right). The reference plane is set at the site of the superficial femoral artery occlusion. The three proximal planes and one distal plane are used to quantify the degree of vascularization.

Figure 5. Coronal and sagittal MIPs obtained after subtraction of pre-contrast images from post-contrast images for consecutively obtained MRA images of the calf in a 35-year old healthy male volunteer. Note presence of collaterals marked by arrows.

Figure 6.

Coronal and sagittal MIPs obtained after subtraction of pre-contrast images from post-contrast images for consecutively obtained MRA images in a participant with occlusion of the superficial femoral artery. Note presence of arteries counted marked by red circles. (a) Coronal image with a reference plane at the highest grade stenosis, three proximal and one distal to it, (b) sagittal image, (c) axial image of the 3rd of the 5 transverse planes shown in (a) with visualized arteries annotated, and (d) sagittal plane with visualized arteries annotated.

Figure 7.

Box-plots showing differences between PAD (PAD) and normal participants (Norm) of a. transfer constant (Ktrans) (min^{-1}), b. capillary perfusion measured by calf muscle plasma volume, Vp (%), c. peak hyperemic popliteal flow (ml/s), and d. time-to-peak flow after cuff release (seconds). All differences between PAD and normal participants was significantly different ($p < 0.05$).

Table 1

Table shows the used scanner, field strength and radiofrequency coil for imaging in each of the 9 CCTRN clinical sites.

Clinical Center	Scanner	Field Strength
Indiana University	Siemens TrioTim	3T
Minneapolis Heart Institute	Siemens Avanto	1.5T
Orlando Regional Health Center	Siemens Sonata	1.5T
Stanford University	GE Discovery	3T
Texas Heart Institute	Philips Ingenia	3T
University of Florida	Siemens Avanto	1.5T
University of Minnesota	Siemens Aera	1.5T
University of Louisville	GE Signa HDxt	1.5T
University of Miami	GE Signa HDxt	1.5T

Table 2

Reproducibility of DCE and flow parameters.

	Reader 1		Mean Difference	ICC	Reader 2	Mean Difference *	ICC
	Obs 1	Obs 2					
Capillary perfusion, measured by plasma volume Vp (%)	4.1±3.6	4.6±4.4	-0.5±1.8	0.90	5.7±4.0	1.2±2.6	0.78
Peak hyperemic popliteal flow (ml/s)	5.1±6.1	4.9±4.7	0.18±1.78	0.95	4.4±3.9	-0.5±1.21	0.95
Resting flow (ml/s)	2.5±1.3	2.5±1.2	0.02±0.61	0.89	2.4±1.4	-0.11±0.62	0.88
Arterial flow reserve [‡] (ml/s)	2.6±5.6	2.4±4.2	0.16±1.59	0.95	2.1±3	-0.39±1.45	0.92
Normalized arterial flow reserve [§] (ratio)	0.99±1.00	1.04±1.26	-0.04±0.81	0.74	1.03±1.47	0.01±0.33	0.97
Time-to-peak flow (seconds)	155±148	176±156	-21±18	0.88	159±145	17±69	0.89
Ktrans [‡] (min ⁻¹)	0.09±0.06	0.11±0.08	-0.02±0.04	0.79	0.11±0.09	-0.002±0.07	0.61

Primary endpoints highlighted.

* Reflects difference between the reader 1 observation 2 and reader 2.

[‡]Ktrans = Influx constant of skeletal muscle from dynamic contrast-enhanced MRI.

[‡]Arterial flow reserve = peak hyperemic popliteal flow – resting flow.

[§]Normalized arterial flow reserve = (peak hyperemic popliteal flow - resting flow)/resting flow

Table 3

Reproducibility of magnetic resonance angiography parameters.

	Reader 1		Reader 2		Mean Difference [*]	ICC
	Obs 1	Obs 2	Obs 1	Obs 2		
Arteries per plane – thigh	5.8 ± 2.7	5.4 ± 2.6	5.3 ± 2.7	5.3 ± 2.7	0.6 ± 1.0	0.86
Collateral count – thigh	2.1 ± 2.7	2.4 ± 2.6	1.8 ± 1.9	1.8 ± 1.9	0.3 ± 1.3	0.89
Collateral count – calf	1.5 ± 1.9	1.1 ± 1.5	0.9 ± 1.5	0.9 ± 1.5	0.6 ± 0.9	0.85
Total collateral count	3.6 ± 3.6	3.4 ± 3.0	2.8 ± 2.8	2.8 ± 2.8	0.9 ± 1.5	0.82

Primary endpoint highlighted

^{*} Reflects difference between the reader 1 observation 2 and reader 2.

Table 4

Test-retest reproducibility of perfusion, flow and magnetic resonance angiography parameters.

	Exam 1	Exam 2	Mean Difference	ICC
Capillary perfusion, measured by plasma volume Vp (%)	5.1±2.2	5.6±2.7	-0.5±1.8	0.73
Peak hyperemic popliteal flow (ml/s)	13.5±6.1	13.5±7.0	-0.03±4	0.85
Resting flow (ml/s)	6.6±2.8	6.2±2.9	0.4±0.9	0.94
Arterial flow reserve $\ddagger$ (ml/s)	6.9±4.2	7.3±4.9	-0.4±3.4	0.74
Normalized arterial flow reserve $\ddagger$ (ratio)	1.14±0.68	1.20±0.66	-0.06±0.71	0.43
Time-to-peak flow (seconds)	35±68	17±7	18±70	0.79
Ktrans* (min ⁻¹)	0.12±0.06	0.13±0.08	-0.01±0.05	0.76
Arteries per plane right thigh	5.6 ± 2.7	6.6 ± 2.8	-0.96 ± 2.25	0.64
Arteries per plane left thigh	6 ± 2.6	6.7 ± 3.1	-0.77 ± 2.21	0.68

Primary endpoints highlighted

* Ktrans = Influx constant of skeletal muscle from dynamic contrast-enhanced MRI.

 $\ddagger$ Arterial flow reserve=peak hyperemic popliteal flow –resting flow. $\ddagger$ Normalized arterial flow reserve = (peak hyperemic popliteal flow - resting flow)/resting flow.

Table 5

Comparison of perfusion, flow and magnetic resonance angiography parameters across PAD and normal participants.

	PAD (n=82, unless noted)	Normals (n=16)	P-value	Age, gender & field strength adjusted
Age	66±9	52±5	<0.001	
Gender (% male)	74.4	68.8	0.24	
Capillary perfusion, measured by plasma volume Vp (%)	3.8±3.1	5.6±2.7	0.031	0.074
Peak hyperemic popliteal flow (ml/s) [†]	3.8±3.5	13.5±7.0	<0.001	<0.001
Resting flow (ml/s) [†]	2.2±2	6.2±2.9	<0.001	<0.001
Arterial flow reserve [‡] (ml/s) [†]	1.59±2.2	7.3±4.9	<0.001	<0.001
Normalized arterial flow reserve ^{//} (ratio)	0.91±1.06	1.20±0.67	0.29	0.49
Time-to-peak flow (seconds) [§]	130±119	17±6	<0.001	0.002
Ktrans (min ⁻¹) [*]	0.07±0.05	0.13±0.08	<0.001	<0.001
Arteries per plane at thigh	5.9±3.1	5.6±1.7	0.71	0.90

Primary endpoints highlighted

^{*} Ktrans = Influx constant of skeletal muscle from dynamic contrast-enhanced MRI.

[†] n=80

[‡] Arterial flow reserve=peak hyperemic popliteal flow –resting flow.

[§] n=75

^{//} Normalized arterial flow reserve = (peak hyperemic popliteal flow - resting flow)/resting flow.