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Oxygenation and flow in the limbs: Novel methods to characterize peripheral artery disease

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Abstract

Peripheral arterial disease (PAD) affects approximately 8 million Americans and is associated with high morbidity and increased mortality. Current therapies for PAD are limited and development of new therapeutic agents is needed. Present diagnostic methods for PAD are insensitive to the subtle microvascular and metabolic changes that occur beyond macrovascular stenosis and therefore may be less useful endpoints for clinical trials. Phosphorus-31 magnetic resonance (MR) spectroscopy, MR muscle perfusion, and MR oximetry are novel methods capable of evaluating both the macrovascular and microvascular changes that occur in PAD patients.

Keywords

peripheral arterial disease; magnetic resonance imaging; spectroscopy; perfusion; skeletal muscle

Introduction

There are 8 million people with PAD in the U.S¹. Recent data in a general population over 40 demonstrated an incidence of PAD defined as ankle brachial index (ABI) of <0.9 of 4.3%². The prevalence is age-dependent, rising to 14.5% in those over 70². In populations at risk including diabetics or smokers, the incidence is nearly 30%³. The annual rate of cardiovascular (CV) events including myocardial infarction, stroke, and CV death is 5–7%⁴. The adjusted risk of dying of a CV event is 2-fold higher than those without PAD⁵. A decline in ABI of more than 0.15 over a 10 year period is associated with an increased risk of all-cause mortality and mortality from CV disease⁶.

PAD is one of the manifestations of a complex systemic atherosclerotic process that involves genetic factors, lipid disturbances, platelet activation, thrombosis, endothelial dysfunction, inflammation, oxidative stress, vascular smooth muscle cell activation, altered matrix metabolism and remodeling⁷. The classical symptom is intermittent claudication, defined as leg pain on walking that usually affects the calf and is relieved by rest⁸. Claudication is insensitive for PAD since it is only present in 11% of patients³ and can be a nonspecific finding^{9–11}. The impact of PAD on quality of life is comparable to the impact of

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Disclosure

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other forms of cardiovascular disease¹². Symptoms of claudication are typically attributed to decreased oxygen supply. However, histopathologic studies have shown evidence of mitochondrial structural and DNA damage which suggests that impaired energy utilization plays an additional role in skeletal muscle dysfunction¹³. Hence tools capable of assessing endothelial function and muscle energetics have been of particular interest in the development of new PAD diagnostic methods.

Current PAD therapy consists of lifestyle modification, structured exercise programs, lipid lowering agents (statins), antiplatelet agents (aspirin, clopidogrel, cilastazol), and percutaneous or surgical revascularization. Treatment effects are modest and their mechanisms are poorly understood. Development of newer therapeutic agents that would increase tissue perfusion may prove beneficial for symptoms relief and limb salvage. Traditional PAD diagnostic methods, such as the ankle-brachial index (ABI) and angiography, are insensitive to microvascular changes of PAD.

We discuss novel non-invasive MR diagnostic methods capable of quantifying skeletal muscle energetics, microvascular function, and muscle tissue perfusion. With the incremental availability of MRI, these novel methods sensitive to the end-organ pathophysiological alterations seen in PAD may prove more accurate, reproducible, and practical endpoints for clinical trials of new PAD therapies, such as angiogenic agents or stem cells.

Assessment of PAD with MR: General Principles

Skeletal muscle resting perfusion and metabolic activity are extremely low and differences between normal volunteers and PAD patients are barely measurable. As such, testing of vascular function and energetics requires a physiological challenge. This can be done with exercise or cuff occlusion. During an exercise paradigm patients typically perform plantar flexion exercise using an MR-safe pedal ergometer which selectively increases flow and metabolic activity in the muscles used in the exercise. During a cuff occlusion paradigm a blood pressure cuff is inflated in the proximal thigh to well above the individual's systolic blood pressure causing tissue ischemia and hypoxia. The cuff is kept inflated for 5 minutes and then rapidly deflated. Following deflation there is generalized reactive hyperemia to all leg muscle groups. The degree of exercise induced flow or reactive hyperemia following cuff occlusion is dependent on large vessel patency, collateral recruitment, endothelial and microvascular function.

Skeletal muscle energetics by Phosphorus-31 MR spectroscopy (³¹P MRS)

³¹P MRS allows *in vivo* measurements of the relative tissue concentrations of phosphocreatine (PCr), adenosinetriphosphate (ATP), and inorganic phosphate (P_i) in an imaging slab¹⁴. PCr is the main energy reservoir and energy transport molecule of skeletal muscle¹⁵. At the start of exercise, PCr is hydrolyzed via the creatine kinase pathway to synthesize ATP. The regeneration of PCr at the cessation of exercise occurs exclusively within the mitochondria and depends on the cell's oxidative phosphorylation capacity and oxygen supply^{15, 16}. Hence exercise is used for ³¹P MRS testing. Multiple kinetic parameters can be characterized before, during and after exercise. The PCr recovery time constant, a monoexponential fit of PCr levels versus time (beginning at the end of exercise), has been the most studied metric of mitochondrial function in PAD.

Several ³¹P MRS studies have shown that the PCr recovery time constant is significantly prolonged in PAD patients compared to normal volunteers regardless of the exercise protocol used^{13, 17–21}. Pipinos et. al. tested 12 PAD patients and 14 normal volunteers using low workload isometric exercise¹³. They found that PCr and adenosinediphosphate (ADP)

recovery time constants of PAD patients were significantly longer compared to normal volunteers ($p < 0.05$). Using symptom limited exercise our group has shown that PCr recovery time constant clearly differentiates PAD patients from normal volunteers with a receiver operator curve (ROC) area of 0.925 ± 0.045 ¹⁸. Additionally, the PCr recovery time constant was highly reproducible in both normal volunteers and PAD patients with an intra-class correlation coefficient (ICC) of 0.95. With graded exercise Greiner et. al found that in addition to PCr recovery time constant, PCr time constant during exercise and the change in PCr concentration from baseline to peak exercise were also significantly increased in the symptomatic (ABI 0.44–1.18) versus asymptomatic (ABI 0.77 – 1.21) legs of PAD patients ($p < 0.009$)¹⁹. The only difference between asymptomatic limbs and normal volunteers was delayed PCr time constant at peak workload ($p < 0.01$). These studies found direct correlation between PCr kinetics and the run-off resistance of PAD patients determined by MRA, but only weak or absent correlation with the screening ABI^{18, 19}.

Skeletal Muscle Perfusion

The primary MR methods to assess skeletal muscle perfusion are first-pass contrast enhanced (CE-MRI) and arterial spin labeling (ASL) MRI. Both techniques provide spatially and temporally resolved measures of skeletal muscle perfusion. First-pass CE-MRI uses a T1-weighted sequence to visualize contrast enhanced blood flow through tissue. ASL has been validated with venous occlusion plethysmography²². It is a contrast-free method that magnetically labels protons in arterial blood which then create changes in signal intensity at the imaging slice. Both techniques can be used with exercise and cuff-occlusion.

First-pass CE-MRI can be used to calculate skeletal muscle perfusion on a ml/100g/min basis using a two compartment model which has been validated in animal studies of myocardial flow using microspheres as a reference technique^{23, 24}. Thompson et. al. used a modified first-pass CE-MRI method with cuffocclusion²⁵. Contrast was administered 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. Although no direct validation or limits of agreement analysis were done and only one PAD patient was studied, the experiments yielded flow rates comparable to other techniques. This method would be limited to a cuff occlusion paradigm since contrast has to equilibrate in the arterial blood pool prior to hyperemia.

Using first-pass CE-MRI our group developed the concept of a perfusion index (PI), defined as the slope of the tissue time-intensity curve (TF) divided by the slope of the arterial time-intensity curve (AIF) from the feeding artery, as a measure of skeletal muscle perfusion²⁶ (Figure 1). In a study of 11 PAD patients and 22 normal volunteers we found that the PI at peak exercise was significantly lower in PAD patients compared to normals ($p < 0.001$). The difference remained significant even in a group of normal volunteers who matched the PAD patient's lower workload ($p < 0.02$). The method proved effective to differentiate PAD patients from normal volunteers with an ROC area of 0.88 (95% CI 0.71 – 0.96) and 0.95 (95% CI 0.77 – 0.99) when including all normal volunteers and only volunteers who performed maximum workload respectively. Inter-observer agreement was high, with an ICC of 0.91. In a follow up study, we found that PI is reproducible when studies were performed on separate days with an ICC 0.60²⁷. The same study showed that perfusion reserve, defined as the exercise TF/rest TF, was preserved in PAD and not useful to differentiate PAD patients from normal volunteers, due to extremely low rest values both in patients and volunteers.

ASL also provides flow measurements on a ml/100g/min basis. In a study of 40 PAD patients and 17 age-matched controls using continuous ASL and cuff occlusion, Wu et. al. found a direct correlation between PAD severity, peak hyperemic flow and TTP hyperemic flow²⁸ (Figure 2). The TTP hyperemic flow appears to be affected with early changes in ABI, whereas peak hyperemic flow was preserved until the more advanced stages of the disease. Our group has studied the performance of pulsed ASL using exercise in 15 PAD patients and 15 normal volunteers²⁹. We found that mean peak exercise flow was significantly greater in normal volunteers compared to PAD patients ($p < 0.001$) (Figure 3). Once again, the difference remained significant when a predetermined group of normal volunteers matched PAD patient's workload ($p < 0.002$). Twelve studies were repeated in 7 PAD patients and 5 normal volunteers. The ICC was 0.87 (95% CI 0.61 – 0.96) and 0.96 (95% CI 0.84 – 0.99) for repeated studies and inter-observer reproducibility respectively.

Blood oxygen level dependent (BOLD) MRI

BOLD MRI is sensitive to the paramagnetic deoxyhemoglobin concentration, which results in a T2* signal loss in the area of interest. Both exercise and cuff-occlusion can be used to study the BOLD signal of PAD patients. During cuff-occlusion there is T2* signal loss and during reactive hyperemia or exercise, there is T2* signal gain due to increased oxyhemoglobin content³⁰. Thus changes in BOLD signal offer an indirect measure of tissue perfusion and have been correlated to skeletal muscle perfusion by relaxometry³¹ and arterial spin labeling (ASL) MRI³².

Using cuff occlusion Lederman et. al. measured the maximum change in T2* ($\Delta T2^*_{\max}$) signal and the time to peak (TTP) maximum T2* signal in the calf of PAD patients and age-matched controls³³ (Figure 4). They found that the $\Delta T2^*_{\max}$ was significantly lower in PAD patients compared to the age matched controls ($p < 0.001$). They also noticed a significantly longer TTP in PAD patients ($p < 0.001$). There was a good correlation between TTP and the ABI. With one exception, all PAD patients with ABI < 1 had a TTP > 50 seconds. The $\Delta T2^*_{\max}$ has to be interpreted carefully since it is greatly influenced by O₂ extraction rate³⁴ and blood volume effects³². It is thought that due to lower flow rates, oxygen extraction is higher in PAD patients, therefore causing lower T2* signal gain. This is further accentuated by decreased blood pool within the region of interest (ROI) analyzed since PAD patients have decreased capillary density³⁵. On the other hand, TTP is more specific and minimally affected by factors other than changes in perfusion^{31, 32}.

The inter-scan and inter-observer reproducibility of BOLD MRI was studied by Versluis et. al. in 8 PAD patients and 10 normal volunteers using cuff occlusion³⁶. Again, TTP was significantly prolonged in PAD patients compared to normal volunteers, but there was no difference in $\Delta T2^*_{\max}$. Their results revealed that TTP was reproducible with an ICC of 0.80 and 0.69 for PAD patients and normal volunteers respectively. The TTP inter-observer ICC was 0.78 and 0.59 for PAD patients and normal volunteers respectively. The $\Delta T2^*_{\max}$ had similar inter-scan reproducibility (ICC of 0.71 and 0.79 for PAD patients and volunteers respectively) but much better inter-observer reproducibility (ICC of 0.99 for both patient groups). The study was limited by inclusion of large vessels in the analyzed ROI which can lead to inflow artifacts.

Susceptibility-weighted Magnetic Resonance Oximetry

Intravascular blood oxygen saturation can be quantified by MR oximetry using any one of three techniques sensitive to the volume fraction of deoxyhemoglobin: T2-³⁷, T2*-³⁸ or magnetic susceptibility-weighted imaging (SWI)³⁹. SWI oximetry is a calibration-free, T2*-sensitive method that uses both magnitude and phase information³⁹. The magnetic susceptibility of blood is determined by the volume fraction of the paramagnetic

deoxyhemoglobin in red blood cells³⁹ and scales linearly with $(1 - \text{HbO}_2)$. Under normal conditions tissue oxygen debt created by periods of ischemia due to arterial occlusion is followed by reactive hyperemia and increased oxygen uptake⁴⁰. As such, repeated measurements of venous oxygen saturation following a period of hypoxia reflect the rate at which deoxygenated blood is replaced, which depends on endothelial function.

Langham et. al. measured the change of oxygen saturation in the femoral/popliteal artery and vein during post-occlusion reactive hyperemia in 10 young healthy (YH) volunteers, 12 PAD patients and 8 age-matched healthy (AHC) volunteers⁴¹. To assess the contribution of the duration of hypoxia to the characteristics of reactive hyperemia two occlusion periods of 3 and 5 min were used. Three parameters characterizing different temporal phases of reactivity were derived: washout time, defined as the time to lowest venous oxygen saturation; upslope, the slope from lowest saturation level to peak saturation level; and overshoot, peak oxygen saturation level achieved after washout (Figure 5). PAD patients had longer washout time ($p < 0.0001$) and lower upslope ($p = 0.0008$) compared to normal volunteers. Neither washout time nor upslope differed significantly between the two healthy subject groups of different ages. The only difference observed with longer occlusion time was a higher overshoot in YH compared to AHC ($p = 0.0116$). The reproducibility of SWI oximetry in PAD patients has not been rigorously studied, although it had a coefficient of variation of 5% in two healthy volunteers tested on three different days at three separate locations along the femoral vessels⁴².

Gaps and Limitations

Multiple novel noninvasive MRI modalities that utilize measures of microvascular function and metabolism are available for the assessment of PAD (Table 1). Patients with contraindications to MR such as claustrophobia or MR-unsafe implants cannot be assessed with these methods. Furthermore, patients with renal impairment (chronic kidney disease [$\text{GFR} < 30 \text{ ml/min/1.73 m}^2$] and those with acute kidney injury) cannot be studied with gadolinium-based CE-MRI due to risk of nephrogenic systemic fibrosis⁴³.

All methods appear to differentiate PAD patients from normal volunteers, although ROC analyses have only been determined for PCr recovery and PI. PCr recovery time constant, peak exercise PI, mean peak exercise pulsed ASL flow, and post-occlusion hyperemia TTP T2*BOLD signal have been shown to be reproducible in PAD patients and normal volunteers. Reproducibility testing has not been carried out with continuous ASL or SWI in PAD patients. Further testing is needed to determine the optimal physiological stress (exercise versus cuff-occlusion) and imaging modality combination.

While exercise testing is more physiological, the resulting peak flow is influenced by subject effort. Thus test-retest and inter-subject variability may be higher. However, we have shown that even when normal volunteers match PAD patients' workload, both PCr recovery and PI remain significantly different between the groups^{18, 26}. This suggests that effective therapeutic interventions could be detected even at low workloads. On the other hand, a cuff-occlusion paradigm is less variable by design and may provide better reproducibility. However, not all MR methods can be tested with both stress modalities, i.e. CE-MRI step-input can only be obtained with cuff occlusion.

Differences in the correlation between physiological parameters and functional testing are yet another indication that these methods test different aspects of PAD. In fact, in our laboratory PCr recovery time constant did not correlate with PI, indicating uncoupling of metabolic activity and perfusion⁴⁴.

Furthermore, our group has seen improvements in PCr kinetics after percutaneous transluminal intervention without improvements in PI in a small cohort of 10 patients⁴⁵. No improvement in PI or PCr recovery kinetics was seen with low density lipoprotein cholesterol lowering with various regimens⁴⁶. A quantitative perfusion method may be more sensitive to detect treatment effects that have been detected by PCr recovery kinetics.

Conclusion

All these parameters offer significant potential for demonstrating benefits of novel therapies including angiogenic agents and stem cells. However, additional investigations are needed to better define their performance characteristics and prognostic value. To date, none of these methods have been used in adequately powered intervention studies to determine their prognostic value for clinically meaningful outcomes. Further studies are needed to determine the parameter that will best detect therapeutic effects. Given the heterogeneity of PAD pathophysiology, the optimal protocol may require a comprehensive assessment involving more than one of these parameters.

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Figure 1.

The left image is a scout image demonstrating the placement of the arterial input function (AIF) slice and the tissue function (TF) slice. The top middle image demonstrates the saturation recovery AIF slice at peak exercise with a ROI placed in the feeding artery. The bottom middle image shows the TF slice at peak exercise with 2 ROI's placed in the anterior tibialis and soleus in regions of peak signal enhancement. Representative AIF and TF curves are shown in the graph of time vs. signal intensity on the right. The perfusion index (PI) is obtained by dividing the slope of the TF divided by the slope of the AIF.

Figure 2.

Schematic illustration of the ischemic-hyperemic paradigm and the flow indexes measured by Wu et. al. Imaging data was acquired continuously from the start of cuff occlusion until 3 minutes after cuff deflation. TTP: Time-to-peak hyperemic flow; PHF: Peak hyperemic flow. Reproduced with permission from Elsevier (Wu et. al. *J Am Coll Cardiol.* 2009;53:2372-7).

Figure 3.

Representative pulsed ASL images of mean hyperemic flow at peak exercise from a normal volunteer (A) and a PAD patient (B). Peak perfusion is measured by placing a ROI in the calf muscle group with greatest signal intensity. Large vessels are excluded from the ROI. At peak exercise, flow is typically increased in the anterior (arrow) and superficial posterior (arrow head) compartments.

Figure 4.

The middle panel illustrates representative regions of interest for anterior tibialis (1), peroneal (2), soleus (3) and medial gastrocnemius (4) muscles on a corresponding T1-weighted image. Panels labeled 1 – 4 illustrate normalized T2* signal time course (BOLD response) during 360 seconds of reactive hyperemia in PAD patients (n=17; black) and age-matched healthy volunteers (n=11; gray) for individual calf muscles. BOLD: blood oxygen level dependent; PAOD: peripheral arterial occlusive disease. Reproduced with permission from Wolters Kluwer Health (Lederman et. al. *Circulation*. 2006;113:2929-35).

Figure 5.

Schematic illustration of the ischemic-hyperemic paradigm and intravascular oximetry parameters measured by Langham et. al. Washout time: time to lowest venous oxygen saturation; Upslope: slope from lowest saturation level to peak saturation level; Overshoot: peak oxygen saturation level achieved after washout. Reproduced with permission from Elsevier (Langham et. al. J Am Coll Cardiol. 2010;55:598-606).

Table 1

Summary of novel CMR modalities to assess PAD.

Imaging Modality	Preferred Stress	Field Strength (T)	Special equipment and software	Validation	ROC analysis for differentiating normals vs. PAD	Reproducibility Testing	
						Inter-observer	Inter-scan
³¹ P MRS(18)	- Exercise	1.5/3	- ³¹ P coil - Comm. software	N/A	+	Not done	+
First pass perfusion(26,27)	- Exercise - Cuff – occl.	1.5/3	- Gadolinium contrast - Comm. software	N/A	+	+	+
Step-input perfusion(25)	- Exercise	1.5	- Gadolinium contrast - Comm. software	Correlated to reference values	Not done	Not done	Not done
ASL(22,29)	- Exercise - Cuff – occl.	3	- CASL: Comm. software - PASL: Comm. software	Venous Pletismography	Not done	- (CASL) + (PASL)	- (CASL) + (PASL)
BOLD(36)	- Cuff – occl.	1.5	- Comm. software	N/A	Not done	+	+
SWI Oximetry(41,42)	- Cuff – occl.	3	- Inv. software	Correlated to reference values	Not done	Not done	(+)*

PAD – peripheral arterial disease; ³¹P MRS – Phosphorus 31 magnetic resonance spectroscopy; ASL – Arterial spin labeling; CASL – Continuous ASL; PASL – Pulsed ASL; BOLD – Blood oxygen level dependent MRI; SWI – Susceptibility weighted imaging; Cuff-occl. – Cuff-occlusion; Comm. software – Commercially available software; Inv. software – Investigator developed software; ROC – receiver operator curve; (+) modality has been shown to discriminate normals vs. PAD patients or reproducible;

* – normal volunteers only.