Assessment of Tissue Perfusion in the Lower Limb
Current Methods and Techniques Under Development
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Peripheral arterial disease (PAD) affects 27 million individuals in Europe and North America.1 This condition causes a severe restriction of blood flow that can lead to critical limb ischemia (CLI), a condition characterized by rest pain, ulceration, or gangrene, which is associated with limb loss in ≈25% of cases.2

The ischemic limb can be revascularized by endovascular techniques (angioplasty/stenting) or surgical bypass. Imaging modalities, including duplex ultrasound, MR angiography, computer tomographic angiography, or intra-arterial angiography, are used to assess the extent of disease, plan intervention, and confirm the patency of major blood vessels after intervention. These techniques do not provide information about perfusion at the tissue level.

An early relatively crude method for assessing ischemic limbs, venous occlusion plethysmography3 relies on the principle that the volume of the limb is dependent on the arterial inflow when venous drainage is occluded. Under resting conditions, ≤70% of blood flow to the limb is directed to skeletal muscle, with the remainder to the skin circulation.4 Venous occlusion plethysmography provides a global indication of limb perfusion but gives no anatomic information and cannot delineate segmental perfusion deficits. It is influenced by numerous factors, such as ambient temperature and arteriovenous shunting that may lead to erroneous readings.

Objective measurement of limb perfusion would particularly benefit patients with CLI but most attempts at addressing this have used patients with claudication. The latter can lead to CLI but is a less severe form of PAD that is often treated conservatively and is characterized by pain only when walking as opposed to pain at rest or gangrene. An accurate, noninvasive method of measuring limb perfusion would aid the diagnosis and treatment of PAD and could be combined with conventional angiography to provide both functional and anatomic information. Some think, for example, that targeted revascularization of specific crural vessels that supply the ischemic/gangrenous area of the limb (ie, using the angiosome concept) would achieve better healing than simply restoring in-line flow to the foot via any available vessel.5 Accurate assessment of segmental tissue perfusion would help to delineate areas of ischemia, to determine the severity of perfusion deficits when there is diffuse rather than focal disease of the larger vasculature, to guide interventions and provide an objective means of measuring improvements accurately.

Medical conditions, such as diabetes mellitus, an important cause of limb loss and functional disability, affect the microcirculation, as well as the larger vessels.6 Conventional imaging does not allow reliable assessment of the microcirculation, and could, therefore, underestimate the degree of functional limb ischemia if the main vessels are patent. Similarly, the effect of the collateral circulation (an important compensatory mechanism for maintaining limb perfusion when the main vessels are occluded) cannot be quantified.7 An accurate method for assessing the functional effect of collaterals on tissue perfusion would be invaluable for determining the efficacy of novel treatments, such as angiogenic cell therapy,8 that aim to promote collateralization.

Here, we provide a critical review of the current and emerging technologies for the assessment of limb perfusion (Table).

Current Techniques

Laser Doppler
Laser Doppler flowmetry (LDF) and laser Doppler imaging (LDI) measure the speed and concentration of blood cells (blood flux and not blood flow; Figure 1) and, therefore, cannot be used to compare perfusion in different individuals.9

LDF requires direct contact and is only effective over a relatively limited area (≈1 mm).3 LDI assesses a larger area of skin from a distance using a scanning motion that incorporates the entire lower limb and foot.10 The wavelength of laser, speed of scanning, and distance between the scanner and the leg affect tissue penetrance and must be kept constant to allow meaningful comparison of serial measurements.10

LDF/LDI was first used in the 1980s to assess the severity of ischemia in PAD, but these modalities only identify ischemic

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Table. Advantages and Disadvantages of Various Modalities for Assessing Perfusion

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<th>Imaging Modality</th>
<th>Pros</th>
<th>Cons</th>
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<td>VOP</td>
<td>Noninvasive</td>
<td>Global indicator of limb perfusion, no anatomic information, no ionizing radiation, performed at the bedside</td>
</tr>
<tr>
<td>LDF/LDI</td>
<td>Noninvasive</td>
<td>Low-depth penetration of laser light (marker of skin perfusion), no ionizing radiation, performed at the bedside</td>
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<tr>
<td>TcPo2</td>
<td>Noninvasive</td>
<td>Marker of skin perfusion, no ionizing radiation, performed at the bedside</td>
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<tr>
<td>PET</td>
<td>Absolute quantification of perfusion representing gold standard for perfusion measurements</td>
<td>Exposure to ionizing radiation limiting serial measurements, significant cost and expertise required, short half-life of radionuclide tracers requiring manufacture near PET imaging facilities</td>
</tr>
<tr>
<td>CEUS</td>
<td>Noninvasive</td>
<td>Operator dependent, no ionizing radiation, can provide anatomic information for perfusion in different muscle groups, easily accessible</td>
</tr>
<tr>
<td>MRI: ASL, BOLD, and DCE</td>
<td>Noninvasive, no ionizing radiation</td>
<td>Low signal:noise ratio (ASL), easy access to imaging facilities, quantifies perfusion in different muscle groups (ASL and DCE), uses endogenous contrast agents (ASL and BOLD), good spatial and temporal resolution (BOLD)</td>
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Transcutaneous Oxygen Pressure

The measurement of transcutaneous oxygen pressure (TcPo₂) is a noninvasive technique that allows longitudinal assessment of local oxygen diffusion from the capillary bed through the skin epidermis and provides an indication of the amount of oxygen delivered to the tissue. TcPo₂ measurements can reflect the severity of PAD, assess ulcer healing potential, and determine the optimal level for limb amputation. TcPo₂ <20 to 30 mm Hg is consistent with CLI, and levels <40 mm Hg are associated with poor healing after amputation. Inhalation of 100% oxygen would normally result in TcPo₂ >100 mm Hg, but in patients with CLI the TcPo₂ does not rise >30 mm Hg. Oxygen therapy resulting in <10 mm Hg rise in limb TcPo₂ is associated with an >60% chance of an amputation at that level not healing. A rise of >40 mm Hg in TcPo₂ after limb revascularization is considered significant and increases the chances of tissue healing.

TcPo₂ measurements vary in different tissues and under different physiological conditions. Barriers to diffusion, such as edema, increased oxygen consumption secondary to inflammation, and vasoconstriction, may lead to falsely low TcPo₂ readings falsely. Readings are also affected by temperature, with a 1°C reduction in the thermistor temperature resulting in a 2% to 3% lower TcPo₂ reading.

Indocyanine Green Fluorescence Angiography

This technique has been used for many years to image retinal vessels, and more recently in the assessment of microanastomoses in surgical flap reconstruction surgery, detection of arteriovenous malformations, and measurement of hepatic blood flow. Indocyanine green is a fluorescent dye that is activated by near-infrared laser light at 780 nm. This allows a penetration of 3 mm from the surface of the skin to assess the subdermal microcirculation. A semi-quantitative measure of perfusion is obtained by measuring pixel fluorescence intensity in different areas of the image from the limb. Perfusion measured by indocyanine green fluorescence angiography correlates with LDI in the hindlimb ischemia model and predicts the likelihood of amputation healing in man. This technique can identify patients with peripheral arterial occlusions who have extensive collateralization. Lack of differences between the 2 groups when ankle-brachial pressure index has been used as the discriminator suggests that indocyanine green fluorescence angiography may be a more sensitive method for assessing perfusion in the limb than ankle-brachial pressure index.
The main limitation of all of these techniques is that they only provide information on perfusion in superficial tissues. Analysis of skin perfusion misses the muscle compartments, which determines symptomatic and functional limitations in PAD. Changes in skin perfusion in PAD are only reliably detected in the later stages of limb ischemia, whereas disturbances in muscle microcirculation is seen much earlier. Muscle perfusion in patients with end-stage CLI requiring amputation correlates well with skin perfusion measured by TcPo2 and LDF. Reliable measurement of muscle perfusion may, therefore, allow intervention at an earlier stage where a more positive outcome in terms of limb salvage is achievable.

Cutaneous blood flow is highly variable and, therefore, is a poor marker of limb perfusion. Autonomic neural dysfunction, as seen in diabetes mellitus, and the presence of infection, for example, can significantly alter skin perfusion.

Emerging Techniques

Positron Emission Tomography

This imaging modality was originally used to measure perfusion and absolute blood flow to the brain and heart but has also been used to quantify blood flow to the musculature of the leg. Myocardial perfusion, assessed by positron emission tomography (PET), correlates extremely well with measurements made using radiolabeled microspheres in animals, a technique that is considered the gold standard in the laboratory setting. Oxygen, in water for injection or mixed with air/CO2 for inhalation, is currently used in man for imaging perfusion using PET. A small number of PET studies have assessed blood flow, oxygen consumption, and oxygen extraction in the lower limb and shown that patients with PAD have decreased blood flow and oxygen consumption in the affected limb after exercise when compared with the contralateral control leg. After stimulation with exercise or intra-arterial administration of vasodilators, the increase in blood flow (hyperemia) is less pronounced in patients with PAD when compared with young healthy volunteers (Figure 2). These studies must, however, be interpreted with caution because they neither use age-matched controls nor remeasure perfusion after limb revascularization. PET does not offer the same spatial and temporal resolution as MRI, but its close correlation with microsphere-derived perfusion measurements in mouse and canine models of ischemia renders it the gold standard in perfusion imaging. PET requires a lengthy scan time and exposure to ionizing radiation precluding serial investigations. Nitrogen-ammonia has been used extensively as a PET agent for assessment of cardiac perfusion but not for the measurement of muscle perfusion in the lower limb. Nitrogen-ammonia and Oxygen both have relatively short half-lives of 10 and 2 minutes, respectively, which limits their versatility. Oxygen is better suited as a perfusion probe in the lower limb because it is freely diffusible and not affected by metabolism. Fluorine flurpiridaz tracer is an alternative, versatile radioisotope for measuring perfusion because of its longer half-life (110 minutes) and high extraction rate, but its use in the clinical setting is still under investigation.

Contrast-Enhanced Ultrasound

Contrast-enhanced ultrasound (CEUS) with microbubble contrast agents (measuring 2.5 μm in size) can assess muscle perfusion in the lower limb and is particularly useful in the evaluation of patients with CLI. CEUS is non-invasive, has a high temporal and spatial resolution, and allows real-time imaging of perfusion changes induced by pharmacological interventions. CEUS can provide valuable information on the extent of perfusion in the affected limb and help guide intervention decisions. However, the use of CEUS in patients with PAD is still being explored and further research is required to fully understand its clinical applications.
perfusion.37–39 These contrast agents consist of a shell and a gas core, the latter endowing echogenicity to the structure. The outer shell is broken down by the acoustic energy of the ultrasound and eventually metabolized by the body. The composition of the shell determines how long the contrast agent remains in the circulation.

Assessment of perfusion in skeletal muscle by CEUS is performed with a linear ultrasound transducer probe placed around the calf and a region of interest (ROI) selected in which only muscle is seen and bone is excluded. The probes used usually have a depth of 4 cm beneath the skin and are held in place by hand, keeping the ROI in view.37 Microbubble contrast is administered intravenously, either as a bolus injection37 or by slow continuous infusion39 with the assumption that signal intensity is a direct measure of microbubble contrast concentration in the ROI (Figure 3). It is not known which method of administration is superior. Changes in muscle perfusion are provoked to help assess perfusion and flow reserve. This can be done using a cuff inflated around the thigh to suprasystolic pressure to occlude the arterial circulation. The cuff is subsequently deflated producing a reactive hyperemia; alternatively the subject exercises to induce functional hyperemia in the muscle and changes in perfusion. In both scenarios, signal intensity can be used to quantify perfusion (Figure 4), but the stressors to increase muscle blood flow in the lower limbs are more difficult to achieve in patients with CLI when compared with claudicants. Parameters, such as time to peak (TTP) contrast, maximal contrast, areas under time–signal intensity curves, and gradients, have been used to assess perfusion.37,39 TTP, maximal signal intensity, and area under the time–signal intensity curves are lower in patients with PAD when compared with healthy controls.37–39

CEUS has been used to assess collateralization, an important compensatory mechanism in patients with PAD.39 Patients with extensive collateralization (assessed by angiography) have a shorter TTP when compared with those that collateralize poorly.39 In contrast, no difference in ankle-brachial pressure index was detectable between patients with good and poor collateralization.

CEUS shows promise for measuring tissue perfusion because it is cheap, easily available, and does not use ionizing radiation, but it remains to be validated against other gold standard modalities, such as 15Oxygen PET. To date, it has only been used to measure perfusion in the gastrocnemius and soleus muscle groups. Its use for other calf muscle compartments is limited by the presence of bone. As with all ultrasound techniques, CEUS is operator dependent and can be limited by artifacts created by patient movement. Although microbubble contrast agents are safe to use40 and only needed in small doses, they are rapidly lost in the circulation.

MRI
This is a promising modality for simultaneously assessing limb perfusion and the larger vasculature using conventional MR angiography. This noninvasive technique does not require ionizing radiation, thereby allowing serial assessment of the patient.
Sequences that show the greatest promise include arterial spin labeling (ASL), blood oxygenation level dependent (BOLD), and first-pass dynamic contrast-enhanced (DCE) MRI.

**Arterial Spin Labeling MRI**

This technique, developed to measure perfusion of the brain, has been used in the kidney, heart, and more recently, skeletal muscle. ASL uses radiofrequency pulse sequences to place a magnetic tag onto the water component of blood proximal to the ROI. A T1-weighted image acquired in the ROI, after a delay to allow for inflow of tagged blood, is subtracted from a control image with no magnetic tagging. The difference in T1 signal between control and tag image is proportional to blood flow and can be used to construct perfusion maps over a designated time period. ASL MRI can be considered analogous to PET perfusion imaging, but unlike PET, only uses blood as an endogenous tracer.

Different methods of ASL are available for perfusion imaging depending on how the tagging is achieved. Continuous ASL (CASL) involves continuous saturation or tagging of arterial water proximal to the imaging slice. This allows for a steady state in muscle magnetization to be reached, which maximizes signal contrast between the control and tagged images. CASL is hampered by magnetization transfer effects that overestimate perfusion and make multislice imaging more complex. Pulsed ASL avoids this problem by tagging over a spatial region close to the imaging slice in pulses and acquiring the tag image between pulses after a postlabeling delay. The magnetization transfer effect is less of a problem with pulsed ASL because less RF power is needed. There is, however, variable delay between tagging and blood reaching the imaging slice, which makes absolute quantification of perfusion more difficult.

Alternative sequences, including QUIPPS (I & II) and Q2TIPS, have been developed to tackle this problem. Pulsed ASL is also limited by a lower signal:noise ratio when compared with CASL. A method known as pseudocontinuous ASL aims to circumvent this problem using short trains of RF pulses to mimic CASL, improving labeling efficiency and achieving a higher signal:noise ratio when compared with pulsed ASL (Figure 5).

Like CEUS, reactive hyperemia or exercise is required to unmask perfusion deficits with ASL. This technique allows absolute quantification (mL/g per minute) of perfusion in the tissues, as well as measurement of parameters, such as peak hyperemic flow and TTP in a ROI over time. Although ASL has not been validated for the measurement of perfusion in the limb in man, comparison with radiolabeled microsphere measurements in the rat hindlimb ischemia model and in the porcine cardiac model has shown good correlation for perfusion. TTP increases with the severity of PAD, and peak hyperemic flow decreases. Analysis of individual muscle groups in the calf reveals that peak hyperemic flow is preserved in the soleus with increasing severity of PAD when compared with the other muscle groups. This may be explained by the dual blood supply of the soleus, which receives blood from both the peroneal and the posterior tibial arteries. ASL shows that patients with PAD have lower muscle perfusion after exercise when compared with age-matched controls.

ASL provides an objective, quantifiable measure of muscle perfusion without ionizing radiation or gadolinium-based contrast agents but has limitations, including a low signal:noise ratio (because magnetically tagged blood represents only 0.5%–1% of the full tissue signal), relatively long scan times and patient movement causing image artifacts, calculations required to quantify perfusion are complex, vascular artifact from large blood vessels causes erroneous measurements, and transit time (the time taken for blood to travel from the tagging region to the ROI) is an important source of error that can be potentiated by disease in the peripheral arteries. Echo planar imaging provides faster acquisition but is associated with magnetic field distortions, making quantification of the MRI signal complex.

**Blood Oxygenation Level-Dependent MRI**

First developed in 1990 for mapping brain activity, BOLD MRI uses the saturation state of hemoglobin as an endogenous contrast agent. The paramagnetic effect of deoxyhemoglobin causes a decrease in the BOLD signal in T2* gradient echo planar imaging MRI sequences (Figure 6).

As with CEUS and ASL MRI, hyperemia is also required to elicit BOLD signal changes because of the relatively low resting blood flow in skeletal muscle capillary beds. Reactive hyperemia offers a more consistent and easily reproducible method to obtain T2* signal intensity changes when compared with functional hyperemia. BOLD measurements can be taken through a single section of the calf at rest, after thigh cuff inflation (arterial occlusion), during reactive hyperemia and during return to baseline. Figure 7 depicts the typical T2*
values obtained before and after cuff inflation and illustrates
the parameters that can be measured using the curve to deter-
mine oxygenation/perfusion, including minimum T2* during
ischemia (T2*min), TTP, and maximum T2* (T2*max) dur-
during reactive hyperemia.

BOLD MRI has been used to detect changes in muscle per-
fusion in patients with PAD and healthy controls.44,54–56 Patients
with PAD have significantly reduced T2*max and a prolonged
TTP when compared with age-matched healthy controls.54

After angioplasty, the T2* signal intensity returns to base-
line faster and there is a trend toward a higher T2*max value
and faster TTP, when compared with preangioplasty of super-
ficial femoral artery lesions in patients with intermittent clau-
dication,55 indicating that arterial revascularization has an
effect on the BOLD MRI signal.

Changes in BOLD T2* signal intensity correlate with LDF
and TcPo2 (measure skin perfusion) in the ischemic-reactive
hyperemic model56; tissue oxygenation (measured by myoglo-
bain proton MR spectroscopy)57; and perfusion measurements
made using venous occlusion plethysmography adapted for
MRI and ASL MRI.58

Although the source of the BOLD signal in peripheral skel-
etal muscle is not fully understood, it is generally accepted that
the signal is mainly related to tissue oxygenation state. BOLD
MRI, unlike ASL, does not allow absolute quantification of
perfusion but instead relies on ratios in signal changes for
semiquantitative measurements. BOLD does, however, pro-
duce a better signal:noise ratio, which allows superior spatial
and temporal resolution when compared with ASL imaging.

First-Pass Dynamic Contrast-Enhanced MRI

This MRI technique measures cardiac perfusion and has
been adapted for assessing perfusion in skeletal muscle.59
T1-weighted MRI sequences capture the transit of intrave-
nously administered paramagnetic contrast agents, such as
gadolinium through tissues, and measure changes in signal
intensity in the muscle. Mathematical algorithms for tracer
kinetics have been investigated to estimate myocardial blood
flow, but this has yet to be used in the clinical setting.60

A reduction in DCE MRI signal intensity, which corre-
lated significantly with radiolabeled microsphere measure-
ments of perfusion, has been demonstrated in the presence of
ischemia in the rat of hindlimb model.61 In man, DCE MRI
measures detectable changes in muscle perfusion after thigh
cuff-induced arterial occlusion of a healthy limb when com-
pared with the uncuffed contralateral limb.62,63 A small DCE
MRI study, lacking age-matched controls, has suggested that
patients with PAD may have a lower perfusion index than
healthy individuals.64

The most significant limitation associated with DCE MRI is
the need for exogenous gadolinium-based contrast agents with
their inherent risk of nephrogenic systemic fibrosis in patients
with chronic kidney disease.

Kinetic Models Used in Contrast-Based Techniques

Kinetic models are needed to quantify perfusion using con-
trast-based imaging modalities to differentiate between the
tracer in the arterial system and in the tissues. In PET imaging,
15Oxygen is freely diffusible into tissue from the intravascular
space, which means a simple single compartment model can
be applied to quantify muscle perfusion.65 For DCE MRI and
ASL, the modeling is more complex and quantifying perfu-
sion is dependent on deconvolving the arterial input function,
calculated from a major artery proximal to the ROI, from the
tissue concentration. A nonlinear relationship between arterial
input function concentration and signal intensity can introduce
errors. Partial volume, signal truncation, and magnetization
transfer effects can also lead to inaccuracies. Finally, the bolus
transit time, which is dependent on factors such as cardiac out-
put and rate of injection, can also introduce variability.66
Conclusions

An accurate, reproducible, and noninvasive imaging technique capable of providing an objective measure of tissue perfusion in the lower limb would be an invaluable tool for managing patients with PAD. Traditional methods of assessing perfusion such as venous occlusion plethysmography, TcPo₂, and PET each have limitations and are likely to be superseded with modern imaging modalities, such as MRI and CEUS. Such imaging modalities would find use in (1) mapping of areas of poor tissue oxygenation and perfusion to allow informed planning for surgical or endoluminal interventions (eg, selecting a target vessel that would be most likely to reperfuse the most ischemic areas of the limb); (2) rapid assessment of improvements in muscle perfusion/oxygenation after intervention on the affected limb; (3) selective targeting of injections of novel therapeutic agents in the ischemic limb (eg, angiogenic cells and cytokines); (4) diagnosis of poor muscle oxygenation and perfusion in cases where there is uncertainty as to the cause of symptoms and signs in the limb.

Successful validation of these novel imaging techniques would present the opportunity of quantitative assessment of muscle perfusion and may ultimately help to improve limb salvage rates in patients with limb ischemia.

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Disclosures

None.

References


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