Skeletal Muscle Perfusion in Peripheral Arterial Disease
A Novel End Point for Cardiovascular Imaging*

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Peripheral arterial disease (PAD) is characterized by lower limb arterial obstruction due to atherosclerosis. There are over 8 million people with PAD in the U.S at present (1). As a consequence of impaired tissue perfusion, PAD patients can experience pain, diminished exercise capacity, and tissue loss, with some ultimately requiring amputation (2). The presence of PAD is a high risk marker of additional cardiovascular disease as the annual rate of events including myocardial infarction, stroke, and cardiovascular death is 5% to 7% (3). Presently used diagnostic methods include the ankle-brachial index (ABI), pulse volume recordings, duplex ultrasonography, venous plethysmography and angiography by X-ray, computed tomography, or magnetic resonance imaging, all of which have limitations.

The screening ABI is 90% sensitive and 95% specific for PAD (4), but is less sensitive to serial changes with therapies other than revascularization. None of these diagnostic studies, including angiography, are particularly useful for serial studies in clinical trials of medical therapies because of insensitivity for changes beyond the macrovascular level.

Effective medical therapy for PAD in 2008 is limited but includes cilostazol and statins, both of which increase walking time (5,6). A major problem for testing therapeutic approaches for PAD that might improve tissue perfusion, such as exercise (7) or angiogenic gene therapy (8), is the lack of adequate end points for clinical trials, especially for serial assessment. For example, a recent unblinded study of bone marrow mononuclear stem cell therapy showed symptomatic improvement in patients without a measurable change in the ABI (9). Hence there is a need for novel imaging approaches to measure end-organ effects of PAD such as skeletal muscle perfusion and metabolism. This sets the stage for studies, such as that by Lindner et al. (10) in this issue of JACC: Cardiovascular Imaging, that aim to measure end-organ physiology in PAD.

In their study, Lindner et al. (10) studied 26 controls and 39 patients with mild but symptomatic PAD (one-half with diabetes mellitus [DM], mean ABI 0.74 to 0.79). They measured tissue perfusion with contrast-enhanced ultrasound of the gastrocnemius and soleus at rest and after 2 min of plantar-flexion exercise. This approach was previously validated in a canine model of skeletal muscle contraction or adenosine-mediated vasodilation (11). Treadmill exercise testing with measurement of oxygen consumption was also performed. They found that exercise duration decreased stepwise from controls to patients with PAD without DM to those with PAD with DM. Diabetes mellitus and contrast-enhanced ultrasound of the gastrocnemius and soleus were all univariate predictors of disease severity based on time to claudication. In patients with DM, flow and flow reserve were the only predictors of PAD severity. On multivariate analysis, flow and flow reserve added incremental benefit to clinical and other variables for predicting PAD severity. The technique was even able to identify reduced flow in patients with ABI >0.90. Thus, a new quantitative method for measuring exercise-induced flow and flow reserve in the end organ appears promising for detecting even mild PAD.
and in patients with preserved ABI, especially diabetics. This is an exciting development in a field with an urgent need for quantitative measures of tissue perfusion.

Some additional questions are raised by the study of Lindner et al. (10). There appears to be some overlap in the perfusion values for exercise blood flow and flow reserve, especially between controls and PAD without DM. A receiver operator characteristic curve would be helpful to identify just how well this measure discriminates patients from controls. Test-retest reproducibility is not presented. The controls are somewhat younger than the patients with PAD and DM. Older age and female gender may contribute to reduced skeletal muscle perfusion with exercise (12). By design, the patients studied had only mild disease, so feasibility and applicability in patients with more severe disease should be investigated in the future. Angiography did not correlate with perfusion in patients with DM, but weighting the angiogram for inflow versus outflow disease may improve the correlation. It is unclear whether there were differences in flow between muscle groups (gastrocnemius vs. soleus). Magnetic resonance imaging (MRI) studies from our group demonstrate visual and quantitative differences in contrast-induced signal intensity at peak exercise between muscle groups that depend upon how the subject performs plantar-flexion exercise (13) (Fig. 1).

Other noninvasive imaging methods are available to make these types of quantitative measures. Thompson et al. (14) used MRI with administration of contrast immediately following cuff inflation around the upper thigh, 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. Our group has used first-pass contrast-enhanced MRI at peak exercise using a MRI-compatible plantar-flexion ergometer to demonstrate differences in perfusion indexed to arterial input between PAD patients and controls, even when controls are matched for work performed (13). Beyond measuring perfusion alone, $^{31}$P MR spectroscopy can be used to measure phosphocreatine recovery kinetics, a sensitive marker of PAD and a promising marker of clinical severity (15). In a study of 87 patients with PAD, calf muscle perfusion did not correlate with cellular metabolism as determined by phosphocreatine recovery at peak exercise (16). Thus, there is uncoupling between calf muscle perfusion and metabolism, supporting the concept that factors independent of blood flow and intrinsic to skeletal muscle are critical in PAD.

Other MRI techniques that do not require the use of exogenous contrast agents are coming to the fore. Arterial spin labeling is a technique that measures perfusion quantitatively in a spatially and temporally resolved fashion (17). It involves tagging inflowing blood and observing its effect on signal intensity after it enters the imaging plane and was first developed for quantification of cerebral blood flow. Arterial spin labeling measurements have been validated against venous occlusion plethysmography in normal subjects using both cuff occlusion hyperemia and exercise with excellent correlation (18). Another such technique is blood oxygen level-dependent imaging, which measures changes in deoxyhemoglobin concentration on the basis of local magnetic susceptibility changes and spin dephasing. This approach has been compared against laser Doppler flowmetry and transcutaneous oxygen pressure in healthy volunteers with cuff occlusion/hyperemia and shown to have a moderate to good correlation for time course of change (19). The PAD patients demonstrated a reduced $T2^*$ signal increase (blood oxygen level-dependent ef-
fect) and delayed time to peak values compared to age matched controls after cuff occlusion/hyperemia (20).

We are thus reaching a time when advanced imaging approaches will allow quantification of skeletal muscle perfusion in patients with PAD. Contrast-enhanced ultrasound is one such technique and MR perfusion measures are another. The ABI is an excellent screening test that is relatively easy to perform. It has a long track record and excellent prognostic ability and will be difficult to improve upon. Screening is not likely to be the role of these innovative imaging approaches. Instead, they will likely be used as end points for clinical trials and methodologies for serial tracking of response to therapy. It matters little what imaging technique is used as long as it is accurate, reproducible, relatively easy to perform, and applicable in multicenter trials for serial measurement of the effect of novel therapies on skeletal muscle perfusion. Imaging again leads the way.

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