A pilot study of regional perfusion and oxygenation in calf muscles of individuals with diabetes with a noninvasive measure

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Objective: **To assess alterations in the regional perfusion and oxygenation of the calf muscles in individuals with diabetes.**
Methods: **Age-matched individuals with (n = 5) and without diabetes (n = 6) were investigated. Skeletal muscle perfusion, oxygen extraction fraction, and oxygen consumption rate were measured by newly developed noncontrast magnetic resonance imaging (MRI) techniques. The subjects lay supine on the MRI table with their foot firmly strapped to a custom-built isometric exercise device. The measurements were performed at rest and during an isometric plantar flexion muscle contraction.**
Results: **Individuals without diabetes had up to a 10-fold increase in muscle perfusion, 25% elevation in muscle oxygen extraction fraction, and a 12-fold increase in oxygen consumption rate in the calf during the plantar flexion isometric contraction. In patients with diabetes, the increases in these parameters were only up to sixfold, 2%, and sixfold, respectively. Exercise oxygen consumption rate was inversely associated with blood HbA1c levels (r^2 = .91).**
Conclusions: **This is the first study to quantify regional skeletal muscle oxygenation in patients with diabetes using noncontrast MRI and warrants additional study. Attenuation of perfusion and oxygenation during exercise may have implications for understanding diabetic complications in the lower extremities. (J Vasc Surg 2014;59:419-26.)**

Complications affecting the lower extremity, such as ulceration, neuropathy, and structural changes of the foot, are a major cause of morbidity associated with diabetes mellitus (DM). Circulatory compromise to the extremity is known to have a major impact on the development of these complications.[1] However, the effects of DM on the circulatory system and the impact of those changes on the delivery of blood to the tissues of the leg are only crudely understood. It is well recognized that individuals with DM are prone to relatively distinct clinical manifestations of dysfunction of both the macrocirculation and microcirculation. These DM-related changes include calcific atherosclerosis, particularly of the tibial vessels, reduced capillary size, altered endothelial function, and thickening of the capillary basement membranes.[2] However, the underlying mechanisms, and in particular, the relationship between the microvascular disease, macrovascular disease, and the lower extremity complications have not been well defined. In part, this has not been explored because of the lack of reproducible quantifiable measures of end-organ perfusion.

Traditionally, the macrocirculation was assessed by ankle/brachial index, contrast imaging of the vessels, and ultrasound duplex.[3] These assessments are reproducible and clinically valuable to evaluate and plan treatment for stenotic/occlusive disease in the larger vessels. Unfortunately, there does not yet exist a similar clinically valuable technique to assess the microcirculatory changes that may also have an important effect on the functional perfusion of the tissues. A number of noninvasive imaging techniques have been used to assess local skin perfusion or oxygenation (<3 mm depth), such as capillaroscopy,[4,5] thermography,[6] laser Doppler flowmetry,[7] laser Doppler imaging,[8] transcutaneous O_2 tension,[9] or orthogonal polarization spectral imaging.[10] These imaging techniques, however, have relatively low spatial resolution, reliability, reproducibility, and sensitivity, and none has been widely adopted into clinical practice. The techniques also are limited to the skin, and thus do not provide information on regional muscle perfusion or oxygenation, which may have an impact on the function of the leg and foot.[11] Another technique that can assess peripheral microcirculation is contrast-enhanced ultrasound.[12,13] However, it usually provides semiquantitative measurements without any information about oxygen utility.

Magnetic resonance imaging (MRI) is a noninvasive imaging modality that provides excellent soft tissue

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contrast and has the capability for detailed delineation of anatomy, perfusion, and metabolism in skeletal muscle.[14-18] We anticipate that delineation of the lower extremity perfusion with this modality could lead to important insights into the impact of microvascular changes on the development of DM-related complications in the limb. Recently, we have developed a new MRI method to assess skeletal muscle perfusion, also called skeletal muscle blood flow (SMBF), and oxygen extraction fraction (SMOEF). The latter is defined as $([O_2]_{artery}-[O_2]_{vein})/[O_2]_{artery}$ and represents the relationship between skeletal muscle oxygen supply and demand. A lower number represents lower oxygen extraction by the muscle. The feasibility of the measurement was demonstrated in healthy young volunteers.[19] In this pilot study, feasibility for the assessment of regional muscle perfusion and oxygenation is demonstrated in DM patients without known macrovascular disease, in comparison with age-matched healthy volunteers. It is hypothesized that the microvascular changes related to DM result in impairment of the regional skeletal muscle perfusion and oxygenation.

METHODS

Patients. Six healthy volunteers (age, 70 ± 3 years old; body mass index, 32.3 ± 8.9 kg/m^2) and five nonsmoking DM patients (age, 66 ± 5 years old; body mass index, 37.9 ± 7.1 kg/m^2; HbA1c, 7.7% ± 2.0%; type 1, n = 1; type 2, n = 4) were recruited for the measurement of perfusion and oxygenation in the calf muscle. The healthy volunteers were nonsmokers, free of cardiovascular, metabolic, and musculoskeletal diseases, and did not have a history of DM or peripheral neuropathy. These volunteers were screened by a questionnaire regarding their history and symptoms. For the DM patients, the mean duration of DM was 7.4 ± 7.3 years. Three DM patients (type 1, n = 1; type 2, n = 2) had peripheral neuropathy, determined by the inability to sense the 5.07 Semmes-Weinstein monofilament and no vibration perception <25 volts measured at the plantar great toe. None of them had a history or current plantar ulcer. All of the DM subjects had a history of cardiovascular disease (4 with hypertension, 2 with cardiac artery bypass graft, and 2 with a myocardial infarction), but none of the subjects had a documented history of peripheral vascular disease. Activity level and exercise capacity were not measured in these groups of subjects but based on previous study of a similar subject population; we expect that the DM subjects had lower physical performance compared with the healthy controls.[29] The local human study committee approved this study, and signed consent forms were received from all volunteers prior to the imaging sessions.

Data collection. Subjects were instructed to not consume alcohol or perform any moderate to heavy exercise 24 hours prior to the imaging session. Each subject was positioned supine on the MRI table with his or her right foot firmly strapped to a pedal of a custom-built isometric exercise device.[19] The resistance of the pedal to depression was adjusted on an individual basis (mean resistance force, 67.7 ± 6.5 N) to allow the subject to completely depress the pedal for the duration of the scan (up to 6 minutes).

Prior to the exercise study, a phase-array cardiac coil was placed between the knee and the heel to cover the lower legs. A three-dimensional noncontrast MR angiography was then acquired,[20] which was used to detect any hemodynamically significant stenosis (≥50%) in large peripheral vessels (anterior tibial artery, posterior tibial artery, and peroneal artery). This approach was established recently with superior sensitivity and specificity.[21] The MRI perfusion and oxygenation measurements were subsequently performed at rest and during a sustained isometric contraction of the plantar flexor muscles, which included the gastrocnemius and soleus muscles. Specifically, each measurement started at 2 minutes after the start of contraction, resulting in a total contraction time of approximately 3 minutes for a perfusion measurement and 6 minutes for an oxygenation measurement (see Imaging Methods section). There were few reports about the steady-state time for muscle perfusion and oxygenation during an isometric contraction. It was estimated that this steady-state time for leg muscle perfusion can be reached after 1 to 2 minutes of muscle contraction.[22] There was a 5-minute rest interval after exercise measurements.

Imaging methods. All images were acquired on a Siemens 3T Trio whole-body scanner (Siemens Healthcare, Malvern, Pa). A study using the methods here was reported recently in healthy young human subjects.[31] To measure SMBF, an arterial spin-labeling method was adapted for skeletal muscle imaging.[23] The two-dimensional arterial spin-labeling sequence parameters included: gradient-echo acquisition; repetition time (TR)/echo time (TE), 2.8/1.2 ms; 10 T_1-weighted images for each T_1 measurement; flip angle, 5°; field of view (FOV), 160 × 112 mm^2; data acquisition matrix, 128 × 90; data average, 3; acquisition time, 50 seconds. A single transverse slice was scanned in the middle of the calf muscle.

Skeletal muscle oxygenation is represented by both SMOEF and the oxygen consumption rate ($SMVO_2$). The latter can be determined using Fick's law: $SMVO_2 = [O_2]_a \times SMOEF \times SMBF$. The constant $[O_2]_a$ is defined as the total oxygen content of arterial blood, and a value of 8.61 μmol × mL^{-1} was used for this pilot study.[24] The $SMVO_2$ provides an accurate measure of total oxygen metabolism in skeletal muscle. The MRI method for SMOEF measurement is based on a method developed for the brain, which relies on the magnetic susceptibility effect of intravascular deoxyhemoglobin.[25,26] A multislice two-dimensional triple-echo asymmetric spin-echo sequence was employed to acquire source images for SMOEF measurements.[36] The imaging parameters are: TR, 4 sec; $TE_1/TE_2/TE_3$, 44/62/80 ms; FOV, 160 × 140 mm^2; data acquisition matrix size, 64 × 56 and interpolated to 128 × 112; single slice, slice thickness, 8 mm; total acquisition time, 3 minutes 48 seconds.

The three-dimensional noncontrast angiography uses images acquired during systole and diastole to remove the venous signals so that arteries can be clearly depicted.