Peripheral microvascular response to muscle contraction is unaltered by early diabetes but decreases with age

Jill M. Slade,1,2,3 Theodore F. Towse,1 Ved V. Gossain,4 and Ronald A. Meyer1,2

Departments of Radiology, 1Physiology, 2Osteopathic Manipulative Medicine, and 4Medicine, Michigan State University, East Lansing, Michigan

Submitted 5 January 2011; accepted in final form 25 July 2011

Slade JM, Towse TF, Gossain VV, Meyer RA. Peripheral microvascular response to muscle contraction is unaltered by early diabetes but decreases with age. J Appl Physiol 111: 1361–1371, 2011. First published July 28, 2011; doi:10.1152/japplphysiol.00009.2011.—Long-term or untreated diabetes leads to micro- and macrovascular complications. However, there are few tests to evaluate microvascular function. A postcontraction blood oxygen level-dependent (BOLD) magnetic resonance imaging (MRI) technique was exploited to measure peripheral microvascular function in diabetics and healthy controls matched with respect to age, body mass index, and physical activity. Postcontraction BOLD microvascular response was measured following 1-s maximal isometric ankle dorsiflexion in individuals with diabetes mellitus type I (DMI, n = 15, age 33 ± 3 yr (means ± SE), median diabetes duration = 5.5 yr) and type II (DMII, n = 16, age 45 ± 2 yr, median duration = 24 yr); responses were compared with controls (CONI and CONII). Peripheral macrovascular function of the popliteal and tibial arteries was assessed during exercise hyperemia with phase contrast magnetic resonance angiography following repetitive exercise. There were no group differences as a result of diabetes in peripheral microvascular function (peak BOLD response: DMI = 2.04 ± 0.38% vs. CONI = 2.08 ± 0.48%; DMII = 0.93 ± 0.24% vs. CONII = 1.13 ± 0.24%; mean ± SE), but the BOLD response was significantly influenced by age (partial r = −0.384, P = 0.003), supporting its sensitivity as a measure of microvascular function. Eleven individuals had no microvascular BOLD response, including three diabetics with neuropathy and four controls with a family history of diabetes. There were no differences in peripheral macrovascular function between groups when assessing exercise hyperemia or the pulsatility and resistive indexes. Although the BOLD microvascular response was not impaired in early diabetes, these results encourage further investigation of muscle BOLD as it relates to peripheral microvascular health.

Functional magnetic resonance imaging (fMRI) may provide a measure of peripheral microvascular function. A transient increase in blood oxygen level-dependent (BOLD) MRI signal intensity is elicited within skeletal muscle after performing brief exercise (e.g., single, brief contraction; Refs. 8, 18, 33). The phenomenon of exercise-induced hyperemia in skeletal muscle is well accepted for repetitive exercise (22) and single contractions (34); the increase in blood flow during exercise occurs within seconds in healthy individuals (43, 55). The MRI-measured BOLD change in skeletal muscle (small vessels) shows a similar transient increase following contraction (33, 48). Muscle BOLD changes arise primarily from an increase in small vessel oxygen saturation and blood volume (47), and therefore the magnitude and time course of BOLD may be useful indicators of small vessel reactivity or recruitment reflecting peripheral microvascular function. Muscle BOLD imaging has good temporal resolution (1 s; MRI pulse repetition time = 1 s; Ref. 33), and therefore the magnitude as well as onset of small vessel reactivity can be evaluated. Skeletal muscle BOLD may prove highly useful because it is a more sensitive index of peripheral vascular function compared with peak large artery functional hyperemia in young, healthy individuals (48). Muscle BOLD is increased in endurance trained individuals (48), and preliminary data suggest it is altered with age (49). Surprisingly, the hemodynamic changes occurring with single contraction stimuli have rarely evaluated in specific populations or disease states, including diabetes.

Compromised vasodilation in the large vessels of diabetics has often been reported, reflected in reduced flow-mediated dilation and hyperemia (3, 10, 24, 30, 37, 39, 45) but not always (46, 56, 61). Long diabetes duration, overt vascular dysfunction, poor glycemic control, and/or omission of important peripheral vascular factors make it difficult to determine how early and the extent that vascular health is compromised during the progression of diabetes. In particular, physical activity is seldom controlled. Far less is known about the development and onset of microvascular dysfunction with diabetes. Recently, with the use of contrast enhanced ultrasound, patients with frank microvascular disease showed compromised capillary recruitment during exercise (61); however, the group with early and uncompromised microvascular health had similar capillary recruitment compared with healthy controls. Interestingly, data from rodent diabetes models show impaired insulin stimulated in the presence of preserved contraction-induced capillary recruitment (57, 58). The existing data reveal differences in microvascular function within the diabetic population and encourage further exploration of the onset of microvascular function with diabetes.

The primary purpose was to employ a novel test of microvascular reactivity in individuals with early diabetes using
muscle fMRI to determine the effect of diabetes on small vessel peripheral vascular function. The secondary purposes were to determine the effects of diabetes on peripheral macrovascular function and determine the influence of known vascular determinants (age, body mass index, physical activity, and glycemic control) on peripheral micro- and macrovascular function.

MATERIALS AND METHODS

Subjects

Subjects with diabetes mellitus type I (DMI) and type II (DMII) and healthy matched controls (CONI and CONII) were recruited from the Lansing Michigan community and the Sparrow Diabetes Center. Within the diabetic population, recruitment targeted individuals with a short duration of diagnosed diabetes, e.g., 10 yr or less. Controls were matched to diabetics on an individual basis for sex, age, body mass index (BMI), and physical activity. Controls were free from chronic diseases including metabolic and cardiovascular diseases. Physical activity levels were measured using the 7-day physical activity recall questionnaire, and scores are reported in kilocalories per kilogram per day (17, 42). All subjects were ambulatory and free of peripheral arterial disease as assessed by ankle brachial index (ABI) at rest >0.90, symptom free of claudication during walking and after the laboratory exercise (self reported), and appearance of retrograde flow at rest in the tibial arteries. Before participation, subjects gave informed written consent; the study was approved by the Michigan State University’s Committee on Research Involving Human Subjects and Sparrow Hospital’s Institutional Research Review Committee.

Habituation Session

Each subject participated in two experimental sessions. On the first visit, subjects visited the Sparrow Laboratory for a blood draw from the antecubital vein to determine glycosylated hemoglobin (HbA1c). Next, after lying supine on an exam table for 10 min, the subjects had their blood pressure, ABI, and manual muscle strength of the dorsiflexors measured. Each subject was instructed on how to perform the two exercise protocols. Blood pressure was measured immediately following dynamic exercise (n = 3) and did not increase, suggesting the dynamic exercise did not elicit systemic changes in blood pressure.

MRI Session

During the second visit, subjects underwent a series of MR acquisitions. To limit the potential effects of food and caffeine on blood flow (15), subjects refrained from eating ≥3 h before their visit and from consuming any caffeinated beverages 6 h before their scheduled visit; only low-fat meals or snacks were encouraged in the 12 h preceding the test. In addition, subjects were instructed not to take aspirin or ibuprofen the 12 h preceding the visit or exercise on the day of the testing due to the effects on blood flow (15). Compliance to these restrictions was verified by asking the subject about food intake, medication, and exercise before testing. The subjects were then prepped with electrodes for an ECG for the cardiac gating of blood flow during MRI. A series of MRIs was acquired primarily to determine muscle BOLD microvascular responses following brief contractions and large artery macrovascular function at rest and following dynamic exercise. In addition, images were acquired to determine muscle size, relative metabolic response, and intramuscular fat content of the leg.

All MRIs were acquired using a standard clinical extremity coil (transmit/receive quadrature coil) on a 1.5 T GE Horizon system (GE Medical Systems, Milwaukee, WI). Subjects were supine in the imager for ~20 min before MRI scanning. The subject’s right foot was secured to a custom-built foot device using a nylon strap with Velcro closures. The force system consisted of a load cell (model SSM-EV-250; Interface, Scottsdale, AZ) mounted to the underside of the footplate. Force during the isometric and dynamic exercise was digitized (model DI-195B; DATAQ Instruments, Akron OH), sampled at 60 Hz, and recorded on a personal computer.

T1-weighted images [3-Plane, TR 100 ms, TE 1.6 ms, 24-cm field-of-view (FOV), 5-mm slice thickness, 11 slices per plane, 256 × 128 acquisition matrix, and 1 NEX] were acquired to locate the largest cross-sectional area (CSA) of the ankle dorsiflexors (anterior compartment) in the right leg. The localizer was always followed by this (ordered) protocol, which is described in more detail below: echo planar imaging with brief isometric contraction (microvascular function), resting T2-weighted anatomical imaging of the leg (muscle size), time of flight flow imaging (to prescribe slices for macrovascular function), resting flow phase contrast angiography imaging, dynamic dorsiflexion exercise, postexercise flow phase contrast angiography imaging (run twice, contiguously for macrovascular function), and postexercise T2-weighted imaging (relative metabolic response).

Peripheral microvascular function was assessed by measuring BOLD changes in the dorsiflexors following maximal isometric contractions. One-shot gradient-recalled echo-planar (functional) images (TR 1,000 ms, TE 40 ms, 18-cm FOV, 1-cm slice thickness, 62.5-kHz bandwidth, and 64 × 64 acquisition matrix) were acquired from a single axial slice transsecting the largest CSA. Functional images were acquired continuously for 4 min, during which time subjects performed a 1-s duration maximal voluntary isometric contraction (MVC) with the dorsiflexors every 30 s; the footplate angle was fixed at 120° and peak force (N) was measured. The time course of BOLD signal intensity change in the dorsiflexors was computed from manually demarked regions-of-interest, drawn with care taken to exclude any resolved vessels confirmed in the corresponding anatomical image. The peak change in BOLD signal intensity above baseline was calculated over the last five postcontractile transients as was the time-to-peak and half recovery time of the BOLD transient. Because relative torque is positively correlated to the magnitude of the postcontractile muscle BOLD response (33), maximal contractions were performed to minimize differences in peak contractile effort and enhance the muscle BOLD response. Single contraction-induced BOLD responses have good test-retest reliability (coefficient of variation = 5.1%; Ref. 33).

Peripheral macrovascular function was primarily assessed by measuring large artery exercise hyperemia. A set of two-dimensional (2D) gradient-recalled-echo time-of-flight flow images was acquired to identify suitable axial/oblique planes for flow measurements. The 2D-time-of-flight sequence consisted of 92 adjacent axial images (TR 18 ms, TE 4.5 ms, 45° pulse, 16-cm FOV, 1.5-mm slice thickness, 256 × 128 acquisition matrix, and 1 NEX) centered on a region 5 cm below the fibular head. The axial images were used to construct a three-dimensional (3D) representation of the vessels within that region. Based on the 3D image, two parallel slices were chosen, one transsecting the popliteal artery 1–2 cm above the popliteal bifurcation and the second transsecting both the anterior and posterior tibial arteries 1–3 cm below their bifurcation from the popliteal artery. The two slices were prescribed as near as possible to be perpendicular to the axis of the arteries.

Flow velocity images (TR 18 ms, TE 6 ms, 30° pulse, 1-cm slice thickness, 14-cm FOV, 256 × 160 acquisition matrix, and 1 NEX) of the selected slices were acquired in retrospectively ECG-gated CINE mode as described previously (31). This MR flow acquisition method depends on the measurement of the extra phase acquired by spins moving along the direction of a bipolar flow-encoding gradient; the extra phase depends directly on velocity. In this study, the flow-encoding gradient was applied perpendicular to the prescribed slices (i.e., parallel to the vessels), with maximum velocity encoding (VENC, corresponding to ± 180° phase shifts) set at 160 cm/s to
acquire arterial velocities. Retrospective gating of the data acquired over 160 heart beats (total acquisition time 2–4 min, depending on the subject’s heart rate) yielded 32 cardiac-gated flow-velocity and magnitude images per slice. Flow (ml/min) was calculated from the individual velocity images by integrating velocity (cm/s) across the area (cm²) of each vessel as described previously (31). Mean flow in each vessel was then calculated from the average velocity across all 32 images (during the cardiac cycle). Vessel flow was measured immediately before and twice during the recovery period after the subjects performed 2 min of dynamic ankle dorsiflexion exercise. Peak blood flow during exercise was extrapolated assuming an exponential recovery (31). During the exercise, the subject moved the footplate through a 30° range of motion from 120° to 90°. The resistance was applied by rubber tubing and was set to equal ~40% of the subject’s MVC when the footplate was at 90°. Approximately sixty 1-s duration contractions were performed at 0.5 Hz. Peak force (N), tension time integral (N·s), and fatigue (percent force loss expressed relative to initial peak force) were measured during the dynamic exercise. The anatomical images (described below, T2-weighted) allowed for anterior tibial artery flow to be expressed relative to the CSA of the dorsiflexors (ml·cm⁻²·min⁻¹). The waveforms from velocity encoded images were also analyzed for resistive index (RI) [(peak systolic velocity – minimum diastolic velocity)/ peak systolic velocity] and pulsatility index (PI) (peak systolic velocity – minimum diastolic velocity/mean velocity) at rest and postexercise. Vessel diameter was computed from the measured CSA; diameter = 2(√CSA/π). Conductance was calculated as the relative mean flow divided by mean arterial pressure. Heart rate was recorded three times during each flow acquisition, and the average heart rate is reported.

Relative metabolic response was assessed with T2-weighted images (axial fast-spin-echo, TR 1,500 ms, TE 24 ms, echo-train length 4, 256 × 160 acquisition matrix, 16-cm FOV, 1-cm slice, and 1 NEX) acquired before and ~7–8 min following dynamic exercise (following all flow-velocity images). This measure of muscle T2 following exercise represents the degree of relative metabolic stress, reflecting the peripheral aerobic fitness of the muscle (32, 40). T2 of the dorsiflexors is reported as the volume weighted average of five slices. The T2-weighted images were used as anatomical measures of dorsiflexor muscle CSA; the largest slice is reported. The images were also analyzed for leg intramuscular fat over five slices using a standard segmentation algorithm (23). Intramuscular fat is expressed relative to muscle CSA.

Intramuscular fat,% 2.5

Vessel diameter was computed from the measured CSA; diameter = 2(√CSA/π). Conductance was calculated as the relative mean flow divided by mean arterial pressure. Heart rate was recorded three times during each flow acquisition, and the average heart rate is reported.

Relative metabolic response was assessed with T2-weighted images (axial fast-spin-echo, TR 1,500 ms, TE 24 ms, echo-train length 4, 256 × 160 acquisition matrix, 16-cm FOV, 1-cm slice, and 1 NEX) acquired before and ~7–8 min following dynamic exercise (following all flow-velocity images). This measure of muscle T2 following exercise represents the degree of relative metabolic stress, reflecting the peripheral aerobic fitness of the muscle (32, 40). T2 of the dorsiflexors is reported as the volume weighted average of five slices. The T2-weighted images were used as anatomical measures of dorsiflexor muscle CSA; the largest slice is reported. The images were also analyzed for leg intramuscular fat over five slices using a standard segmentation algorithm (23). Intramuscular fat is expressed relative to leg muscle CSA.

Statistics

DM1 and DMII groups were separately compared with their control groups (CONI and CONII). Independent samples t-tests were used to determine group differences in subject characteristics and exercise characteristics as well as for the main outcomes measures (BOLD response, large artery hyperemia, RI, and PI). All controls were also regrouped based on the presence or absence of a family history of diabetes, and group differences in peak muscle BOLD responses were compared with an independent samples t-test. Repeated-measures ANOVA was used to determine differences in large artery blood flow at rest, peak during dynamic exercise, and two times following the dynamic exercise protocol (4 total time points). Peak velocity, vessel diameter, and heart rate were also tested with repeated-measures ANOVA (at rest and twice postexercise). Multiple regression (entering all variables) was used to determine the influence of age, physical activity, BMI, and HbA1c on muscle BOLD response and also the influence of these variables on large artery responses in the anterior tibial artery (relative increase in anterior tibial artery flow, RI, and PI). Violations of sphericity were corrected with Huynh-Feldt degrees of freedom and the Bonferroni procedure was used to correct for multiple comparisons. Significance was set at P < 0.05. Data are presented as means ± SE.

RESULTS

Descriptive Characteristics

Appropriately matched controls were recruited for 15 DMI and 16 DMII. Physical characteristics of the subjects are shown in Table 1. There were no significant differences in age, BMI, or physical activity between the diabetic groups and their respective control groups (e.g., DM1 vs. CONI and DMII vs. CONII). There were no differences in muscle CSA, intramuscular fat or mean arterial pressure, between groups. Resting heart rate was higher for DMII vs. CONII (P = 0.031) and showed a trend to be significantly greater in DM1 vs. CONI (P = 0.075; see Table 1). There was a positive correlation between intramuscular fat and age (n = 62; r = 0.446; P < 0.001) and a trend for a significant correlation between intramuscular fat and physical activity score (n = 62; r = −0.233; P = 0.068).

As expected, the diabetics had significantly greater HbA1c than their control groups (DM1 = 7.7 ± 0.24 vs. CON = 5.2 ± 0.09; P < 0.001; Table 1). DM1 subjects were taking an average of 2.75 medications, most taking a rapid and slow acting insulin, with three also taking kidney protective or antihypertensive medications [one each taking an angiotensin II receptor blocker, hydrochlorothiazide, and angiotensin converting enzyme (ACE) inhibitor]. DMII subjects were taking an average of 5.1 medications. In addition to medications to enhance glucose control [biguanides (75%) and thiazolidin-
Fig. 1. Single contraction exercise and microvascular response in the dorsiflexors. Axial MRI slice of the leg is shown: anatomical image with the dorsiflexor muscles outlined (A) and corresponding echo planar images (B) to assess muscle blood oxygen level-dependent (BOLD) microvascular responses (D and E). Force (C), muscle BOLD signal intensity (SI) with all points displayed (D), and the average BOLD response (E: this is the average transient response from D) are shown for a representative type I diabetic (left), representative type II diabetic (middle), and a type II diabetic with no BOLD response (right).
ediones (50%)), 81% (n = 13) of DMII were taking at least one type of kidney protective/antihypertensive medication [ACE inhibitors (n = 5), angiotensin II receptor blockers (n = 4), calcium channel blockers (n = 2), β-blockers (n = 2), and hydrochlorothiazide (n = 2)].

Known vascular complications were present in two DMI subjects; one individual had neuropathy and retinopathy and the other had neuropathy. Vascular complications were present in four DMII subjects; one with nephropathy, two with neuropathy, and one with cardiovascular disease; the individual with cardiovascular disease reported a single positive stress test suggesting a minor heart block, but subsequent tests were negative. The controls were medication free (CONI) or used an average of 1.1 medications per day (CONII). The groups were comprised of nonsmokers, except for one smoker in DMI (light smoker who also had vascular complications) and one smoker in DMII. The average duration of diagnosed diabetes was ~11 yr (range: 0.5–47 yr) and ~6.5 yr (range: 0.8–28 years) for DMI and DMII, respectively. Seven individuals in DMI and 10 individuals in DMII had been diagnosed with diabetes for <5 yr while seven others had diagnosed diabetes for 5–10 yr, resulting in the majority of the sample (78%) with diagnosed diabetes <10 yr. A family history of diabetes [first (n = 8) or second degree relative (n = 8)] was present in ~50% of controls (7 CONI and 9 CONII). A family history of diabetes was present in 60% of DMI and 80% of DMII.

Peripheral Vascular Function

Peripheral microvascular function. Muscle BOLD responses to single contractions are shown in Fig. 1. Average peak muscle BOLD response was not different between DM and CON groups (P = 0.559; Fig. 2). This was also the case if the subjects with frank vascular disease were removed [peak BOLD = 2.38 ± 0.52 (CONI) vs. 2.30 ± 0.39 (DMI) (n = 26); peak BOLD = 0.91 ± 0.29 (CONII) vs. 0.96 ± 0.28 (DMI) (n = 24)]. The magnitude of the BOLD response varied substantially (peak BOLD response = 1.5 ± 0.18%, range = 0.0–7.3%); BOLD response was influenced by age, BMI, and physical activity (R² = 0.343; P < 0.001). Age showed a significant negative relationship (partial r = −0.384; P = 0.003; see Fig. 3) in the model. Lower physical activity scores and higher BMI were associated with lower BOLD responses; physical activity score and BMI (P ≤ 0.146) showed trends as significant factors in the regression model. For diabetics, there was no significant relationship between the peak BOLD response and HbA1c or peak BOLD response and the number of years with diabetes. The time to reach peak BOLD was similar between DMI and CONI (n = 28) and slightly longer for DMII vs. CONII (P = 0.032; n = 23); the lower subject number is due to the lack of temporal hemodynamics for subjects with no BOLD response. There were no group differences in BOLD half recovery time (DMI = 3.4 ± 0.4 s; CONI = 3.6 ± 0.5 s; DMII = 3.6 ± 0.6 s; CONII = 3.6 ± 0.6 s). The average force during the BOLD imaging protocol was 93.6 ± 0.6% of MVC for all groups during the isometric exercise and did not vary between groups (see Table 2).

Some individuals had no transient BOLD response following a single brief maximal contraction (Figs. 1 and 2). One subject in each of the TYPEI groups and nine subjects in the TYPEII groups (n = 5 DMI; n = 4 CONII) did not have a BOLD response after brief contraction. These individuals were older (49 vs. 37 yr old; P < 0.001) and had higher BMI (35 vs. 30, P = 0.006) compared with individuals with a transient BOLD signal change. Both smokers (one each DMI and DMII), three DM with known microvascular complications (neuropathy) and a DMII who reported heart disease on a subsequent telephone follow-up did not have a muscle BOLD response. Three additional diabetics with neuropathy (n = 1) and retinopathy (n = 3) for whom a control was not recruited also had no BOLD response. In addition, four of the five controls with no BOLD response had a family history of diabetes.

For controls with a family history of diabetes, the peak BOLD response was ~50% lower (BOLD = 1.1 ± 0.2%) [mean (SE)] compared with the controls without a family history of diabetes (BOLD = 2.1 ± 0.5%); the group difference showed a trend for significance (P = 0.08; n = 30; data missing from 1 control; Fig. 4). Four out of five controls...
without a contraction-induced BOLD response had a family history of diabetes (n = 2, primary degree relative; n = 2, secondary degree relative) as noted above. These sub-groups of controls (with and without a family history of diabetes) were similar in mean age [family history, 40 ± 12 yr vs. no family history, 37 ± 12 yr [mean (SD)]], physical activity (family history, 34.2 ± 1.65 kcal·kg⁻¹·day⁻¹ vs. no family history, 33.5 ± 1.7 kcal·kg⁻¹·day⁻¹), and BMI (family history, 31 ± 10 vs. no family history 31 ± 6); they were not, however, recruited as matched pairs.

Peripheral macrovascular function. Velocity-encoded phase-contrast MRIs are shown in Fig. 5. Large artery data are comprised of fewer subjects (n = 48) because of problems associated with cardiac gating or significant movement of the acquisition coil during dynamic exercise (which changed the angle of flow acquisition); this happened in six subjects and also resulted in the removal of data from their matched control. Resting heart rate was greater in both DM groups compared with CON groups (P ≤ 0.035; see Table 2). Heart rate increased nominally from rest to postexercise (by ~1–2 beats/min for each group, P > 0.05), suggesting minimal changes in central hemodynamics. Heart rate was significantly elevated in the diabetic groups compared with their controls at all time points (P < 0.001). Resting flow was not significantly different in the popliteal artery or the anterior tibial artery (Table 3 and see Fig. 6). Likewise, resting vessel diameter was not significantly different between groups (see Table 3).

Dynamic exercise increased popliteal artery flow by approximately fourfold (P < 0.005), primarily increasing blood flow to the dorsiflexors. There were no group differences in popliteal flow over time (Fig. 6). Anterior tibial artery flow increased by ~10-fold for all groups (DMI = 9.4 ± 4.8-fold, CONI = 10.2 ± 5.1-fold, DMII = 9.6 ± 7.4-fold, and CONII = 10.8 ± 6.9-fold) with no significant group differences in flow over time (Fig. 6). The increase in flow over time in the popliteal and anterior tibial arteries was associated with vasodilation [anterior tibial artery (% increase): DMI = 21.4 ± 4.67%, CONI = 20.8 ± 4.11%, DMII = 18.4 ± 3.98%, and CONII = 20.1 ± 3.28%] and increased blood velocity [anterior tibial peak velocity (% increase): DMI = 106 ± 15.0%, CONI = 86 ± 11.4%, DMII = 81 ± 11.4%, and CONII = 82 ± 10.7%; see also Table 3]. On average posterior artery flow was greater after exercise (~38.5% greater flow 2 min postexercise) suggesting an increase in flow during exercise. However, for some individuals (n = 6) posterior tibial artery flow did not increase or slightly decreased following exercise and therefore neither peak flow during exercise or exponential recovery rate

Table 2. Exercise characteristics

<table>
<thead>
<tr>
<th>TYPE I</th>
<th></th>
<th>TYPE II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMI</td>
<td>CONI</td>
<td>DMI</td>
</tr>
<tr>
<td>Isometric exercise</td>
<td>n = 15</td>
<td>n = 15</td>
<td>n = 16</td>
</tr>
<tr>
<td>MVC, N</td>
<td>247 ± 13</td>
<td>240 ± 17</td>
<td>213 ± 19</td>
</tr>
<tr>
<td>Peak BOLD force, N</td>
<td>227 ± 12</td>
<td>220 ± 15</td>
<td>202 ± 19</td>
</tr>
<tr>
<td>Tension time integral, N·s, per contraction</td>
<td>254 ± 21</td>
<td>251 ± 23</td>
<td>213 ± 27</td>
</tr>
<tr>
<td>Dynamic exercise</td>
<td>n = 11</td>
<td>n = 11</td>
<td>n = 13</td>
</tr>
<tr>
<td>Initial force, %MVC</td>
<td>37.6 ± 2.2</td>
<td>40.7 ± 2.8</td>
<td>42.1 ± 3.0</td>
</tr>
<tr>
<td>Fatigue, %initial force</td>
<td>7.4 ± 3.1</td>
<td>13.0 ± 3.9</td>
<td>15.4 ± 2.9</td>
</tr>
<tr>
<td>Tension time integral, N·s, sum of all contractions</td>
<td>4818 ± 395</td>
<td>4897 ± 438</td>
<td>3622 ± 416</td>
</tr>
<tr>
<td>T₂, % change</td>
<td>9.0 ± 0.9</td>
<td>9.5 ± 0.9</td>
<td>8.4 ± 1.1</td>
</tr>
<tr>
<td>Heart rate (rest)*</td>
<td>67 ± 3</td>
<td>59 ± 3</td>
<td>79 ± 2</td>
</tr>
<tr>
<td>Heart rate postexercise,* beats/min</td>
<td>68 ± 2</td>
<td>61 ± 3</td>
<td>80 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Exercise characteristics during dorsiflexion isometric exercise (single contractions) performed during acquisition of blood oxygen level-dependent (BOLD) MRI sequence and for a 2-min bout of dynamic exercise. MVC, maximal voluntary contraction. BOLD force is the average force during functional BOLD imaging. DM groups had greater heart rate at rest and following exercise *P < 0.05, DM > CON.
could be determined. Conductance at rest and following exercise in the anterior tibial artery was not significantly different between groups (Table 3).

There were no differences in large artery function for both the anterior tibial artery when assessing RI or PI at rest (see Fig. 7 and Table 3). RI and PI following dynamic exercise in the anterior tibial artery postexercise were not different between groups (Table 3). Both RI and PI were also not significantly different in the popliteal artery at rest or postexercise (data not shown). Age, physical activity, BMI, and HbA1c did not explain any variance in large artery peak hyperemia, RI or PI; all regression models were insignificant.

The initial force during the dynamic exercise was \( \sim 39 \pm 1.2\% \) for all groups and was not different between groups (Table 2). The exercise bout elicited similar changes in muscle \( T_2 \) (Table 2), suggesting no difference in relative metabolic

### Table 3. Macrovascular measurements at rest and in response to exercise

<table>
<thead>
<tr>
<th></th>
<th>TYPE I</th>
<th>TYPE II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMI (n = 11)</td>
<td>CONI (n = 11)</td>
</tr>
<tr>
<td>Popliteal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>0.58 ± 0.023</td>
<td>0.61 ± 0.026</td>
</tr>
<tr>
<td>Peak velocity, mm/s</td>
<td>294 ± 23</td>
<td>289 ± 15</td>
</tr>
<tr>
<td>Mean flow, ml/min</td>
<td>47.5 ± 2.5</td>
<td>52.6 ± 4.6</td>
</tr>
<tr>
<td>Anterior tibial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>0.33 ± 0.01</td>
<td>0.35 ± 0.01</td>
</tr>
<tr>
<td>Peak velocity, mm/s</td>
<td>243 ± 21</td>
<td>270 ± 21</td>
</tr>
<tr>
<td>Mean flow, ml·cm(^{-2})·min(^{-1})</td>
<td>1.4 ± 0.20</td>
<td>1.4 ± 0.16</td>
</tr>
<tr>
<td>Conductance, ml·cm(^{-2})·min(^{-1})·min(^{-1})</td>
<td>0.015 ± 0.002</td>
<td>0.015 ± 0.002</td>
</tr>
<tr>
<td>RI</td>
<td>1.28 ± 0.023</td>
<td>1.26 ± 0.019</td>
</tr>
<tr>
<td>PI</td>
<td>12.7 ± 1.92</td>
<td>12.9 ± 1.48</td>
</tr>
<tr>
<td>Postexercise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popliteal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>0.64 ± 0.018</td>
<td>0.64 ± 0.022</td>
</tr>
<tr>
<td>Peak velocity, mm/s</td>
<td>419 ± 33</td>
<td>403 ± 23</td>
</tr>
<tr>
<td>Extrapolated mean flow end-exercise, ml/min</td>
<td>246 ± 15</td>
<td>256 ± 23</td>
</tr>
<tr>
<td>Mean flow 2-min postexercise, ml/min</td>
<td>195 ± 13</td>
<td>193 ± 17</td>
</tr>
<tr>
<td>Anterior tibial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>0.40 ± 0.016</td>
<td>0.42 ± 0.014</td>
</tr>
<tr>
<td>Peak velocity, mm/s</td>
<td>475 ± 22.5</td>
<td>491 ± 35.8</td>
</tr>
<tr>
<td>Extrapolated mean flow end-exercise, ml·cm(^{-2})·min(^{-1})</td>
<td>12.0 ± 1.29</td>
<td>14.3 ± 1.86</td>
</tr>
<tr>
<td>Mean flow 2-min postexercise, ml·cm(^{-2})·min(^{-1})</td>
<td>9.5 ± 0.83</td>
<td>9.9 ± 1.09</td>
</tr>
<tr>
<td>Conductance, ml·cm(^{-2})·min(^{-1})·min(^{-1})</td>
<td>0.849 ± 0.098</td>
<td>0.871 ± 0.120</td>
</tr>
<tr>
<td>RI</td>
<td>0.94 ± 0.025</td>
<td>0.94 ± 0.030</td>
</tr>
<tr>
<td>PI</td>
<td>3.53 ± 0.378</td>
<td>3.48 ± 0.347</td>
</tr>
</tbody>
</table>

Values are means ± SE. There were no differences in any resting or postexercise vessel parameter. Mean blood flow postexercise was measured \( \sim 2.5 \) min after the exercise, and the peak extrapolated mean blood flow was calculated assuming a mono-exponential recovery. Popliteal artery flow is expressed in absolute terms (ml/min), whereas anterior tibial artery is expressed relative to the size of CSA (ml·cm\(^{-2}\)·min\(^{-1}\)) of the dorsiflexor muscles. RI, resistive index; PI, pulsatility index.
response between the diabetics and their matched controls. Postexercise muscle T2 was acquired ~7.5 min after the exercise, which was similar between groups.

DISCUSSION

The primary outcome was that contraction-induced peak muscle BOLD response was not independently influenced by early diabetes when key determinants of vascular health were controlled. This was the first attempt to exploit single contraction-induced hemodynamic responses in a population known to develop compromised vascular function. The most intriguing finding was the absence of a BOLD response measured in all groups, particularly in the TYPEII groups. In addition, BOLD was negatively correlated to age. Peripheral macrovascular function (peak flow in the popliteal and anterior tibial arteries) during active hyperemia was also not significantly different between groups as was also the case for RI and PI indicating no effect of diabetes on large vessel outcomes. Lastly, the time to reach peak BOLD response was slower for DMII compared with CONII. According to our recently published modeling, a longer time to peak BOLD is influenced predominantly by slower blood flow kinetics (47). While significantly longer, the time for DMII (6.5 s) is similar to other studies on young, healthy participants (~ 6.3 s) (48).

Our main findings are consistent with recent findings from Womack et al. (61), who showed no difference in large artery or capillary recruitment in patients with uncomplicated diabetes (e.g., no present microvascular disease) with submaximal forearm exercise. Interestingly in the aforementioned study, only patients with nephropathy and neuropathy showed compromised capillary recruitment, which parallels our findings of compromised muscle BOLD (absent muscle BOLD) in several diabetic patients with known microvascular disease. It may be that the redundancy in vasodilatory factors (7, 35) may protect overt losses in microvascular function early in the disease process providing an explanation for why early diabetes showed no microvascular dysfunction.

Are the findings of intact exercise-induced vascular responses at odds with previously reported vascular impairments in diabetes (3, 10, 24, 30, 37, 39, 45, 62)? We suggest the findings are not necessarily contradictory. First, many of the published studies were performed in people that have a long duration of diagnosed diabetes (~ 20 yr (10), ~ 19 yr (11), and ~ 27 yr (30)] have profound secondary vascular complications (11, 30, 37, 53), have poorly controlled blood sugar (30, 59), or fail to implement strict, well-matched control groups (3, 37, 59) and/or account for factors known to influence peripheral vascular function. In particular, diabetes studies focusing on peripheral vascular function rarely measure physical activity, despite the known positive effects of exercise on peripheral vascular fitness (6, 13, 27, 41).

Second, we suggest that the difference in findings lies in the principle mechanisms involved in reactive hyperemia and exercise-induced hyperemia. While NO production and bioavailability play a large role during some protocols (insulin induced blood flow and reactive hyperemia; Ref. 15), it appears to be less important during exercise (see Ref. 50) and in particular following brief single contractions (2). Importantly, insulin-mediated increased flow and capillary recruitment are reduced in insulin-resistant animal models (57) but not in response to exercise (58). These results suggest different hyperemia pathways and also show that microvascular function during exercise may not be impaired with diabetes. Exercise hyperemia was also not impaired in type I diabetic patients (45). On the
Muscle using FMD (25, 26, 54), exercise (8, 9, 18, 33, 48), and contraction-induced responses as they reflect arteriole function. Assessing exercise induced hyperemia and in particular single dilation during reactive hyperemia, there is added value in with what has become a fairly standard use of flow-mediated compromised arteriole function with aging (1, 21). Thus, even blood flow has recently been highlighted in studies showing fact, the importance of the microcirculation in the control of single brief contraction arises principally from rapid arteriole modulation, which is dependent on NO, has rather consistently been necessarily reduced with diabetes, reduced flow-mediated dilation, which is dependent on NO, has rather consistently been reported to be reduced in diabetes (10, 30, 39, 62).

Contrary, others (24) have shown reduced femoral artery peak flow following exercise in diabetics. While peak exercise flow was reduced (24), it was unclear if the change in flow from rest was influenced by diabetes, which could explain the different reported outcomes for exercise induced vascular responses. Lastly, while exercise mediated vascular responses are not necessarily reduced with diabetes, reduced flow-mediated dilation, which is dependent on NO, has rather consistently been reported to be reduced in diabetes (10, 30, 39, 62).

A third main point is that the vascular response following single brief contraction arises principally from rapid arteriole vasodilatation (44, 51, 52) reflecting microvascular function. In fact, the importance of the microcirculation in the control of blood flow has recently been highlighted in studies showing compromised arteriole function with aging (1, 21). Thus, even with what has become a fairly standard use of flow-mediated dilation during reactive hyperemia, there is added value in assessing exercise induced hyperemia and in particular single contraction-induced responses as they reflect arteriole function.

BOLD responses have previously been evaluated in skeletal muscle using FMD (25, 26, 54), exercise (8, 9, 18, 33, 48), and vasodilators (54). The main physiological components of the contraction-induced muscle BOLD response are blood volume and hemoglobin saturation (33, 47). Muscle BOLD responses are influenced by the administration of vasoactive endothelial dependent and independent substances (54) and are decreased following reactive hyperemia in patients with peripheral artery disease (26). Several other factors modulate the BOLD response including blood flow (magnitude and time course) and oxygen consumption (47). In fact, small contraction-induced changes in blood flow likely account for the absence of the BOLD response for a number of individuals in the present study.

Controls with a family history of diabetes had ~50% lower BOLD responses compared with those without a family history. Most of the controls that had no BOLD response also had a family history of diabetes. A family history of diabetes is associated with decreased macrovascular and microvascular reactivity (3, 14, 20) and can occur in the absence of hyperglycemia and insulin resistance (3). A trend towards a significant effect of family history of diabetes on the magnitude of the muscle BOLD response is particularly interesting given the absence of an effect of diabetes. Together these findings suggest decreased vascular reactivity may be causally linked to the development of diabetes.

Muscle BOLD microvascular responses were associated with age, physical activity levels, and BMI, albeit more weakly with physical activity and BMI. Peripheral vascular health is well documented to decline with advancing age (4, 29). Recently, it was shown that the arteriole vasodilatory capacity and rate are reduced with age in rats (1, 21). A small or absent BOLD response has been observed in older adults [76 ± 6 yr (SD)] (49) in agreement with the age effect in the current study. Likewise, the blood flow response in older individuals with brief submaximal forearm contractions is reduced (5). We (48) have previously shown that muscle BOLD is strongly influenced by chronic physical activity; increased vascular volume and/or vascular reactivity may largely influence the BOLD response, evident from their reported increases with endurance exercise (19). In addition, the average peak BOLD response was ~50% lower in the TYPEII groups (DMII and CONII) compared with the TYPEI groups. The older age, higher BMI, and lower physical activity in the TYPEII groups may have partially contributed to the lower BOLD responses for TYPEII groups vs. TYPEI groups. While muscle BOLD was influenced by known determinants of vascular function, large artery peripheral vascular function (peak active hyperemia, RI, and PI) was not influenced by age or physical activity in this patient population, providing additional support that muscle BOLD and perhaps the single contraction-induced hemodynamic response are more sensitive measures of peripheral vascular function.

This study had some limitations. The present study was potentially confounded by the use of kidney protective/antihypertensive medications and thiazolidinediones. Antihypertensive medications, particularly ACE inhibitors, have been shown to improve endothelial function (36) and large artery compliance (60) in diabetes and thiazolidinediones improve endothelial function (38). We chose to test subjects without disruption of medications, with the exception of aspirin and ibuprofen. A washout period before vascular function assessment may provide a better assessment of endogenous vascular function.

Fig. 7. Group averaged cardiac gated waveforms from the anterior tibial artery before and after dynamic exercise protocol. There were no differences between the groups in the pulsatility index or the resistive index calculated from these waveforms. Group means ± SE are shown.
function. On the other hand, type II diabetics are routinely prescribed kidney protective/antihypertensive medications, suggesting that representative type II diabetes groups are also taking these medications. Both approaches have benefits, and it would certainly be valuable to assess vascular health under both conditions; we chose to test subjects without disruption of medications, with the exception of aspirin and ibuprofen. Although some of these medications have been shown to influence NO-mediated vasodilation, including flow mediation (e.g., rosiglitazone), there are limited data suggesting that these medications influence exercise-mediated hyperemia, in particular single contraction-induced hyperemia. Another limitation was the sample size of the study. This may have contributed to marginal correlations. However, even with the small sample sizes within each group, there was no statistical trend supporting a difference in muscle microvascular function between controls and diabetics, suggesting that the study was not underpowered.

In summary, the contraction-induced muscle BOLD response was not diminished in individuals who had a relatively short duration of diagnosed diabetes. However, the utility of muscle BOLD as a noninvasive peripheral microvascular measure should not prematurely be dismissed, as this study only represents a snapshot of individuals with diabetes. Age was associated with muscle BOLD, and several individuals had no muscle BOLD response, including those with known vascular complication and also otherwise healthy individuals, some of whom had a family history of diabetes. It may be that these otherwise healthy individuals proceed to develop diabetes and/or vascular disease. If true, muscle BOLD responses would be extremely important for measuring and predicting microvascular dysfunction. To our knowledge, transient single contraction-induced hyperemia, whether it be large artery flow or small vessel hyperemia/oxygenation changes, has never been evaluated before in patient populations. Single contraction-induced hemodynamic changes, including muscle BOLD, may prove useful in future explorations of vascular health.

ACKNOWLEDGMENTS
We thank the subjects for participating and Malak Saddy for contribution to data analysis.

GRANTS
This work was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grants AR-050203 and AR-043903.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


