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# Abnormal Skeletal Muscle Capillary Recruitment During Exercise in Patients With Type 2 Diabetes Mellitus and Microvascular Complications

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**Objectives** We sought to determine whether skeletal muscle capillary recruitment is impaired in type 2 diabetes mellitus (DM) with and without microvascular complications (MC).

**Background** Insulin and exercise each stimulate recruitment of skeletal muscle capillaries. Insulin-mediated recruitment is impaired in insulin-resistant humans and animals, but exercise-mediated recruitment has not been studied.

**Methods** We studied 20 control subjects, 22 patients with DM, and 8 patients with DM + MC. With the patients under fasting conditions, contrast-enhanced ultrasound perfusion imaging of the forearm flexor muscles was performed to evaluate capillary blood flow and blood volume at rest and during low- or high-intensity contractile exercise (25% and 80% maximal handgrip). Rheologic parameters of erythrocyte deformability and plasma viscosity were measured.

**Results** Muscle capillary responses to exercise were similar between the control and DM groups, but were reduced ($p < 0.05$) in those with DM + MC. The DM + MC group had a ≈50% reduction in capillary recruitment and a ≈60% to 70% reduction in capillary blood flow during both low- and high-intensity exercise compared with the control group. These abnormalities were independent of disease duration. Patients with DM + MC were more insulin resistant than DM patients and had an elevated whole blood viscosity that correlated with plasma glucose ($p = 0.001$) and C-reactive protein ($p = 0.003$).

**Conclusions** Capillary recruitment during low- and high-intensity exercise is normal in uncomplicated type 2 DM but is impaired in those with microvascular complications. Abnormalities in capillary recruitment may be related to abnormal hemorheology, although larger trials are needed to establish this relation. (J Am Coll Cardiol 2009;53:2175–83) © 2009 by the American College of Cardiology Foundation

There is increasing evidence that abnormal skeletal muscle capillary responses in insulin-resistant patients contribute to impaired glucose metabolism and perhaps microvascular complications of type 2 diabetes mellitus (DM). In normal, healthy individuals, physiologic hyperinsulinemia produced by a carbohydrate-rich meal or by a euglycemic clamp stimulates a rapid expansion of skeletal muscle capillary blood volume (CBV) (1–3). Insulin-mediated capillary recruitment is largely nitric oxide (NO)-dependent (4–6). This response is thought to augment glucose uptake by increasing the permeability-surface area product, thereby increasing glucose and insulin access to muscle interstitium. In insulin-resistant patients and animals, CBV and capillary blood flow do not increase normally in response to insulin, which may contribute to abnormal glucose homeostasis (7–10).

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There has been increasing interest in whether capillary responses to exercise, which are not entirely NO-dependent (11–14), also are abnormal in patients with type 2 DM. Patients with type 2 DM have been shown to have lower total oxygen consumption and total body glucose uptake during submaximal exercise compared with healthy subjects (15). There is evidence in humans that these metabolic defects may be secondary, at least in part, to impaired microvascular flow responses (15,16). Under hyperin-

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**Abbreviations and Acronyms**

**CBV** = capillary blood volume
**CEU** = contrast-enhanced ultrasound
**CRP** = C-reactive protein
**DM** = diabetes mellitus
**DM + MC** = diabetes mellitus with microvascular complications
**NO** = nitric oxide
**PI** = pulsing interval
**VI** = video intensity

sulinemic conditions, exercise-mediated augmentation of total skeletal total muscle blood flow is blunted in insulin-resistant patients (16). However, in animal models of advanced DM, changes in flow and CBV in response to muscle contraction appear to be similar to that in healthy controls (17). It is not clear whether the inconsistency of these results is related to species, differences in disease manifestation for single gene-targeted animal models of DM, or differences in exercise intensity. With regard to the latter, regulatory mechanisms of muscle blood flow and the relative contribution of capillary recruitment varies according to the intensity of contractile exercise (2,18).

The aim of this study was to use contrast ultrasound perfusion imaging to determine whether augmentation in CBV or capillary flow in skeletal muscle is impaired during low- or high-intensity exercise in patients with DM. We also tested whether abnormalities in skeletal muscle capillary responses would be more severe in patients with microvascular complications of DM and also whether perturbations in blood rheology, which can influence capillary flow independent of arteriolar vasoregulatory tone (19,20), contribute to flow impairment.

## Methods

**Study population.** The study was approved by the institutional Human Investigation Committee of the University of Virginia. Twenty healthy adult patients and 30 obese (body mass index >30 kg/m$^2$) patients with type 2 DM were studied. Eight of the patients with DM were identified as having microvascular complications defined as proteinuria (>30 μg/mg creatinine) on a spot urine collection within 1 week of the study, or diagnosis of neuropathy made by a neurologist based on summed clinical data (21). Subjects with angina, congestive heart failure, claudication, peripheral vascular disease, an ankle brachial index ≤0.9, or uncontrolled hypertension (>150/90 mm Hg) were excluded. Other exclusion criteria for control subjects included a history of hypertension, dyslipidemia, body weight >10% over ideal, and first-degree relative with diabetes.

**Study design.** At an initial screening visit, the maximal force generated on a calibrated handgrip ergometer was determined for the subject's dominant arm, with the average of 3 attempts used. Urine samples were collected for protein measurement. Three days before the study visit, the subjects discontinued the use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and metformin. They were admitted to the General Clinical Research Center the evening before the study and fasted overnight.

The following morning, blood was drawn for measurement of lipid subfraction analysis by ultracentrifugation, glucose, hemoglobin $A_{1c}$, plasma insulin, C-reactive protein (CRP), blood viscosity, and erythrocyte deformability. The insulin and glucose data were used to calculate the homeostatic model assessment index of insulin sensitivity calculated by: $(I \cdot G)/405$, where $I$ is fasting plasma insulin (μU/ml) and $G$ is fasting plasma glucose (mg/ml). Brachial artery blood flow and skeletal muscle perfusion in the proximal forearm by contrast-enhanced ultrasound (CEU) were measured at baseline, then during both low- and high-intensity exercise.

For low-intensity exercise, subjects performed a 1-s handgrip exercise at 25% of their pre-determined maximal force value every 5 s for 2 min. Handgrip frequency was then reduced to every 20 s, and brachial artery blood flow and skeletal muscle perfusion were measured within 3 min. After a 20-min rest period the imaging studies were repeated with 80% maximal force value every 5 s for 2 min, then every 20 s thereafter at which time brachial artery blood flow and skeletal muscle perfusion measurements were repeated.

**Brachial artery blood flow.** Ultrasound of the brachial artery 5 cm above the antecubital fossa was performed with a linear-array transducer (L7-4 transducer, HDI-5000CV, Philips Ultrasound, Reigate, United Kingdom). Brachial artery diameter and centerline averaged peak velocity at the

**Table 1 Clinical Characteristics and Laboratory Data**

| | Control Patients (n = 20) | DM (n = 22) | DM + MC (n = 8) |
|---|---|---|---|
| Age (yrs), median | 47 | 53 | 54 |
| Sex, M/F | 9/11 | 3/19 | 3/5 |
| Duration of DM (yrs), median (IQR) | — | 2.5 (4.0) | 7.0 (5.0) |
| Hypertension, % | 0 | 36* | 75* |
| Current smoking, % | 10 | 18 | 25 |
| History of dyslipidemia, % | 5 | 68* | 100* |
| Body mass index, kg/m$^2$ | 23 ± 3 | 34 ± 6* | 35 ± 5* |
| Medications, % | | | |
| Aspirin or clopidogrel | 0 | 41* | 63* |
| Beta-blocker | 0 | 0 | 25 |
| ACE-I or ARB | 0 | 41* | 50* |
| Statin | 0 | 55* | 75* |
| Hemoglobin-A1c, % | 5.2 ± 0.3 | 6.9 ± 2.2* | 8.5 ± 2.2† |
| Plasma glucose, mg/dl | 90 ± 15 | 117 ± 60 | 233 ± 82* |
| Plasma insulin, μU/ml | 5.8 ± 4.4 | 16.9 ± 13.2* | 15.0 ± 8.6* |
| HOMA, median (IQR) | 1.2 (0.7) | 3.9 (2.9)* | 6.6 (7.1)* |
| Triglycerides, mg/dl | 90 ± 35 | 135 ± 68 | 273 ± 239† |
| Cholesterol, mg/dl | | | |
| LDL | 118 ± 30 | 106 ± 29 | 113 ± 36 |
| HDL | 55 ± 12 | 48 ± 11 | 40 ± 14* |
| CRP (mg/l), median (IQR) | 0.6 (0.7) | 4.0 (5.9)* | 5.8 (4.2)* |

*p < 0.05 compared with normal control patients; †p < 0.05 compared with control patients and DM; all values are corrected for multiple comparison.

ACE-I = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; CRP = C-reactive peptide; DM = diabetes mellitus; DM + MC = diabetes with microvascular complications; HDL = high-density lipoprotein; HOMA = homeostatic model assessment of insulin resistance; IQR = interquartile range; LDL = low-density lipoprotein.

same vessel location measured with pulsed-wave spectral Doppler and angle correction software. Brachial artery blood flow was measured by the product of the cross-sectional area and time-averaged peak velocity.

**Contrast-enhanced ultrasound.** Harmonic power-Doppler imaging (HDI-5000CV, Philips Ultrasound) was performed at a transmission frequency of 3.7 MHz with a linear-array transducer at a mechanical index of 1.1 to 1.2 and a pulse repetition frequency of 2.5 kHz. The deep forearm flexor muscles were imaged in the trans-axial plane one-third of the distance from the antecubital fossa to the wrist. Lipid-shelled octafluoropropane microbubbles (Definity, Bristol-Myers Squibb Medical Imaging, New York, New York) were infused intravenously at a rate of 0.12 to 0.16 ml/min. Intermittent imaging was performed with the use of an internal timer, and images were acquired at incremental pulsing intervals (PIs) from 1 to 15 s. Video intensity (VI) was measured from a region-of-interest placed over the flexor digitorum profundus and flexor pollicus longus muscles. Averaged frames obtained at a PI of 1 s were digitally subtracted from averaged frames at longer PIs to eliminate signal from the majority of noncapillary vessels with a mean erythrocyte velocity >2.4 cm/s (22). Time versus VI data were fit to the function: $y = A(1 - e^{-\beta t})$, where $y$ is VI at time $t$; $A$ is the plateau VI reflecting relative capillary blood volume, and $\beta$ is the rate constant reflecting the capillary erythrocyte velocity (22,23).

**Blood rheologic parameters.** Erythrocyte deformability and whole-blood viscosity, 2 rheologic factors that strongly influence vascular resistance at the capillary level (19,20), were measured from fasting blood samples. For erythrocyte deformability, 25 $\mu$l of citrated whole blood was placed in 5 ml of polyvinylpyrrolidone (5%) in phosphate-buffered saline. A laser-assisted optical rotational cell analyzer (LORCA, Mechatronics, Amsterdam, the Netherlands) was used to measure the erythrocyte elongation index (ratio

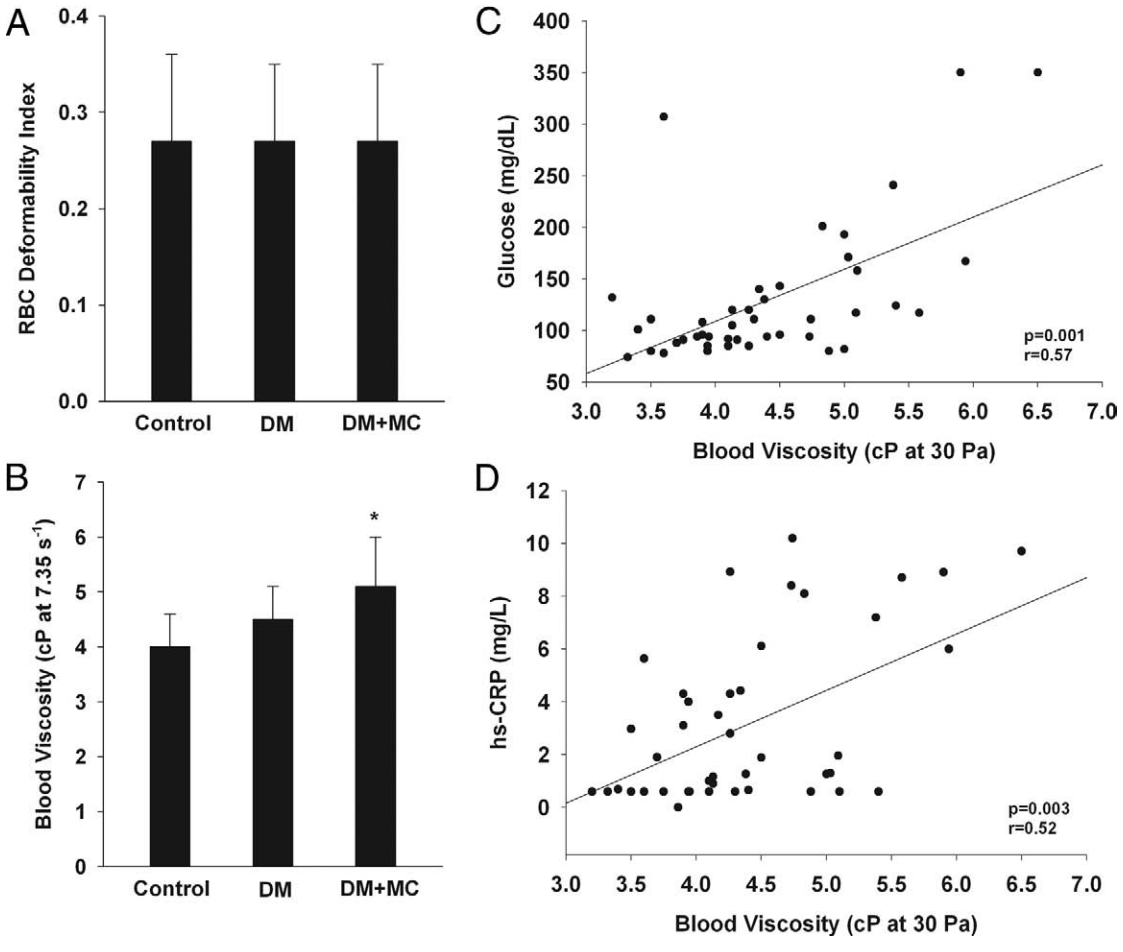


**Figure 1** Blood Rheology Data

**(A)** Erythrocyte deformability (mean ± SEM) measured by the elongation index at 30 Pa in control subjects and in patients with either uncomplicated diabetes mellitus (DM) or diabetes with microvascular complications (DM + MC). **(B)** Blood viscosity (mean ± SEM) measured at a shear rate of 7.35 $s^{-1}$. Correlation between blood viscosity and either **(C)** plasma glucose or **(D)** high-sensitivity C-reactive protein (hs-CRP) peptide is shown. *p < 0.05 versus control (adjusted for multiple comparisons). cP = centipoise; RBC = red blood cell.

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of short- to long-axis dimension) at 37°C and a shear stress of 30 Pa. Whole-blood viscosity at 37°C was measured with a rotational viscometer (EW-98936-00, Cole-Parmer, Vernon Hills, Illinois) at a shear rate of 7.35 $s^{-1}$.

**Statistical analysis.** Data were analyzed on SAS software (version 9.1, SAS Institute, Cary, North Carolina). Clinical characteristics were analyzed by the Fisher exact test or chi-square analysis for frequency or percentage variables; 1-way analysis of variance for normally distributed variables with post-hoc testing of individual comparisons with paired *t* test; or the Wilcoxon rank-sum test for medians. Bonferroni correction was applied for these multiple comparisons. Pearson's correlation coefficients and linear regression were used for the assessment of associations between pairs of continuous variables. Differences in perfusion data between groups and between conditions were analyzed with the mixed model approach to repeated measures incorporating baseline perfusion as a covariate. The covariates triglycerides, viscosity, and CRP were added one at a time to the mixed models. The Tukey-Kramer adjustment for multiple comparisons was used for follow-up comparisons between diagnostic groups.

## Results

**Clinical characteristics.** The baseline clinical characteristics for the 3 study groups are presented in Table 1. Body mass index, HgbA1c, and plasma insulin levels were greater in the 2 DM patient groups compared with healthy control individuals. All DM patients with microvascular complications had proteinuria as a complication criteria (median [interquartile range]: 474 [631] μg/mg vs. 7 [11] μg/mg creatinine for DM + MC and DM cohorts, respectively; $p = 0.0001$) whereas 75% also had a diagnosis of neuropathy. These patients tended to have a longer duration of disease, increased serum triglycerides, and more severe insulin resistance reflected by the median homeostatic model assessment index than other groups. Erythrocyte deformability was similar between cohorts (Fig. 1A), but mean blood viscosity was significantly increased in DM + MC patients compared with the control group (Fig. 1B). Blood viscosity correlated modestly with both plasma glucose and C-reactive peptide (Figs. 1C and 1D) but not with serum triglycerides ($p = 0.51$) or any other plasma lipid measurement.

**Exercise performance and brachial blood flow.** Maximal handgrip force did not vary significantly between groups (median force of 26, 22, and 24 kg for control, DM, and DM + MC subjects, respectively). There was a small (6%) but not statistically significant increase in HR between baseline and maximal exercise measured in approximately two-thirds of patients, which was not different between groups. Brachial artery blood flow (Fig. 2) was similar between groups at baseline and during low- and high-intensity periodic handgrip exercise (25% and 80% maximal force). In all groups, brachial artery blood significantly increased over baseline only at the highest exercise intensity (80% maximal force).

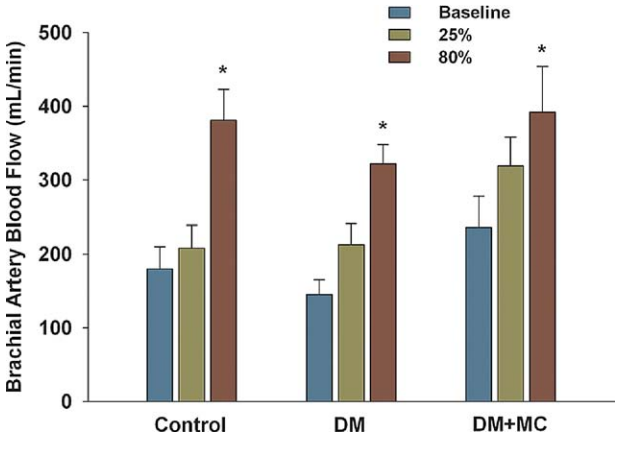


**Figure 2** Brachial Artery Blood Flow at Rest and During Exercise

Flow (mean ± SEM) was derived from 2-dimensional and Doppler ultrasound in control subjects and in patients with either uncomplicated DM or DM + MC. *$p < 0.05$ (adjusted) versus baseline. Abbreviations as in Figure 1.

**Skeletal muscle perfusion.** Illustrated in Figure 3 are background-subtracted CEU images from the proximal forearm flexor muscles and corresponding pulsing interval versus VI data at rest and during low- and high-intensity exercise in a control subject. During low-intensity periodic handgrip exercise, skeletal muscle blood flow (the product of the A- and β-values) increased largely because of an increase in CBV (A-value or plateau intensity), which is consistent with microvascular recruitment as the dominant vascular response. Further increases in blood flow during greater-intensity exercise were secondary to a further increase in CBV with an additional increase in capillary erythrocyte velocity (β-value). Figure 4 illustrates data from a DM + MC patient, whereby blood flow in response to incremental exercise did not increase as much as in the control patient largely because of a blunted CBV (A-value) response.

Data from all healthy control subjects showed a stepwise increase in skeletal muscle capillary blood flow with incremental levels of exercise (Fig. 5A). Similar to previous observations (2), exercise-mediated changes in muscle capillary blood flow surpassed the corresponding changes in brachial artery blood flow (Fig. 2), suggesting some redistribution of brachial artery flow within the limb during exercise. Capillary blood flow responses to exercise in patients with uncomplicated DM were not significantly different ($p = 0.52$) from control subjects (Fig. 5A). Flow responses were, however, impaired in DM + MC patients during both low- and high-intensity exercise ($p < 0.001$), the degree of which was similar for the 2 levels of exercise ($p = 0.59$ for interaction between cohort and exercise level). Abnormal blood flow during exercise in the DM + MC group was attributable in part

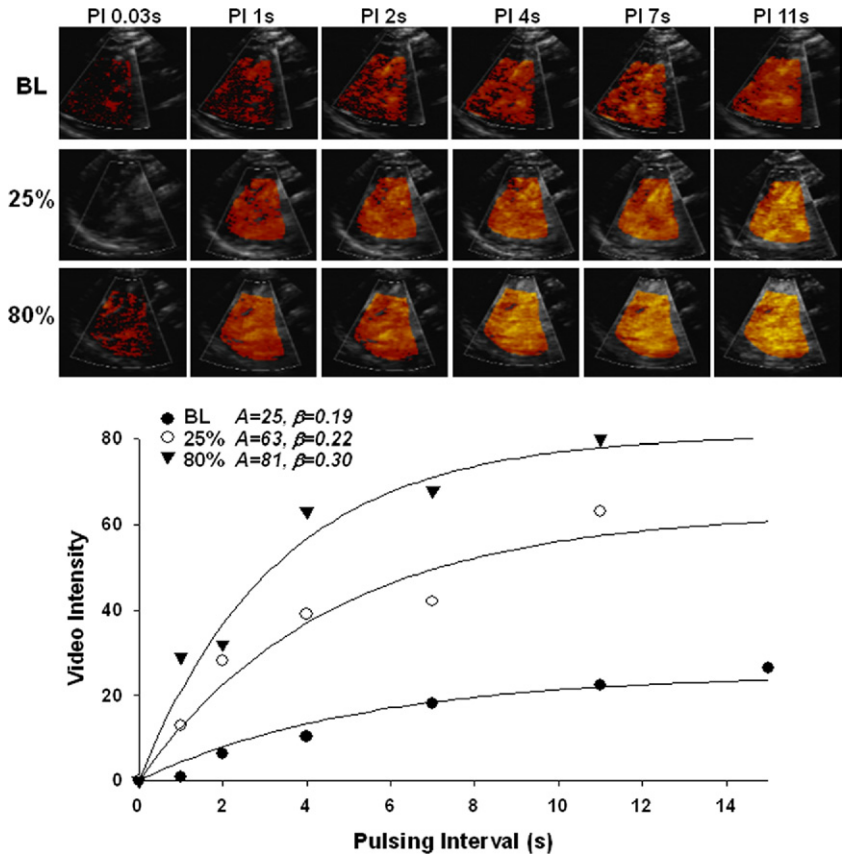


**Figure 3** **Contrast-Enhanced Ultrasound in a Control Subject**

Background-subtracted color-coded images of the proximal forearm flexor muscles, acquired at incremental pulsing intervals (PIs) under baseline (BL) resting conditions and during 25% or 80% maximal grip force exercise, are illustrated at the **top**. Corresponding PI versus video intensity data are shown in the **graph**.

to a blunted CBV response (Fig. 5B), suggesting an impairment in exercise-mediated capillary recruitment. Differences in capillary blood velocity between the control and DM + MC group were small and did not reach statistical significance (Fig. 5C).

Capillary recruitment in response to exercise, expressed as a percent change CBV from baseline, was impaired in DM + MC patients versus healthy control subjects ($p = 0.02$) (Fig. 6). The degree of impairment was similar for low- and high-intensity exercise ($p = 0.91$ for interaction of exercise level and cohort). Because of differences in baseline clinical variables, an analysis also was performed with a 2-cohort comparison (DM and DM + MC). This analysis revealed a significant impairment in flow augmentation ($p = 0.03$) and a borderline impairment in CBV augmentation ($p = 0.06$) during both low- and high-intensity exercise in the DM + MC compared with DM patients that was not influenced by the level of exercise.

**Variables associated with capillary response.** Abnormal capillary responses to exercise in patients within the DM + MC were independent of disease duration. Although triglyceride, viscosity, and CRP values were significantly increased in the DM + MC group and correlated with changes in CBV, none of these factors provided additional predictive information about capillary recruitment in response to exercise once baseline CBV, exercise level, and cohort were accounted for in the model. Differences in the change in CBV between the DM + MC patients and control patients remained significant after adjusting for these factors, suggesting that the differences in CBV response between groups were not due simply to any of these laboratory variables alone.

Although there was a modest correlation between glucose levels and change in CBV ($p = 0.04$ and $p = 0.07$ for 25% and 80% exercise, respectively), adjustment for glucose levels in the model was not possible because of little overlap in glucose levels between the diagnostic groups. Neither history of hypertension nor systolic blood pressure at the time of perfusion imaging correlated with changes in CBV. For the 2-cohort comparison (DM and DM + MC), the incorporation of age, CRP, and serum viscosity in the model improved the overall p value for comparison of flow response and CBV response, although the impact of each of these covariates in the

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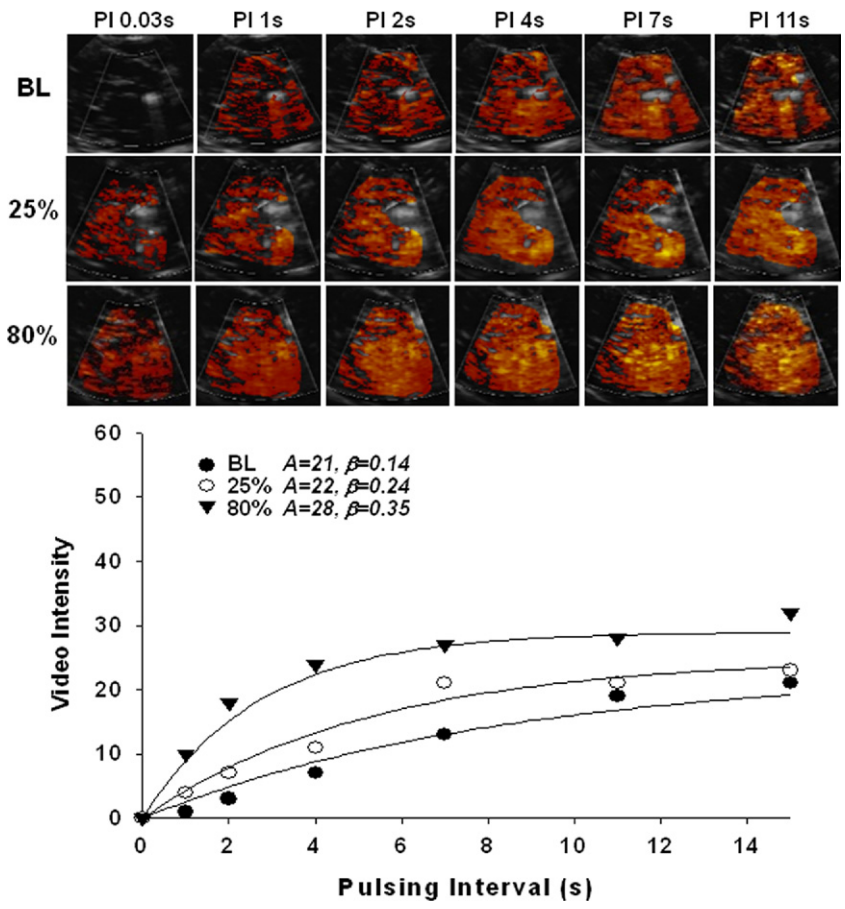


**Figure 4** **Contrast-Enhanced Ultrasound in a Patient With Diabetes and Microvascular Complications**

Background-subtracted color-coded images of the proximal forearm flexor muscles, acquired at incremental PIs under BL resting conditions and during 25% or 80% maximal grip force exercise, are illustrated at the **top**. Corresponding PI versus video intensity data are shown in the **graph**. Abbreviations as in Figure 3.

model did not reach significance except for the influence of age on CBV.

## Discussion

The main findings of this study were that skeletal muscle capillary responses to periodic contractile exercise are preserved in subjects with well-controlled uncomplicated type 2 DM but are impaired in those with microvascular complications. In particular, changes in CBV were abnormal in those with microvascular complications, reflecting an underlying abnormality in microvascular recruitment during exercise. The degree of vascular impairment was similar for different degrees of exercise intensity.

Both insulin and skeletal muscle contractile exercise increase flow and insulin transport in skeletal muscle. There is accumulating evidence that abnormal flow responses may not just be a consequence of DM but may also play a role in abnormal glucose storage and utilization. This idea originated from early studies in which insulin was shown to increase limb blood flow in a dose-dependent fashion (24,25). Inhibitors of NO synthase block this response (4,5). These agents also block insulin-mediated limb glucose uptake, supporting the notion that limb blood flow and glucose storage are coupled (6), although NO also can augment glucose uptake by increasing glucose transporter (i.e., GLUT-4) translocation (25).

Vascular responses at the muscle capillary level have been examined with CEU imaging as well as other techniques, such as capillary xanthine oxidase activity and microdialysis measurement of the capillary permeability-surface area product (3,8,17,22,26). These techniques have independently confirmed that hyperinsulinemia triggers a rapid increase in CBV. Capillary recruitment appears to be the dominant effect at the capillary level because microvascular blood velocity changes little with insulin (8,22). Changes in capillary recruitment are also NO-dependent (6) and are impaired in diabetic animals and obese insulin-resistant subjects (7–9).

The current study examined microvascular responses to contractile exercise to test whether there is a global impair-

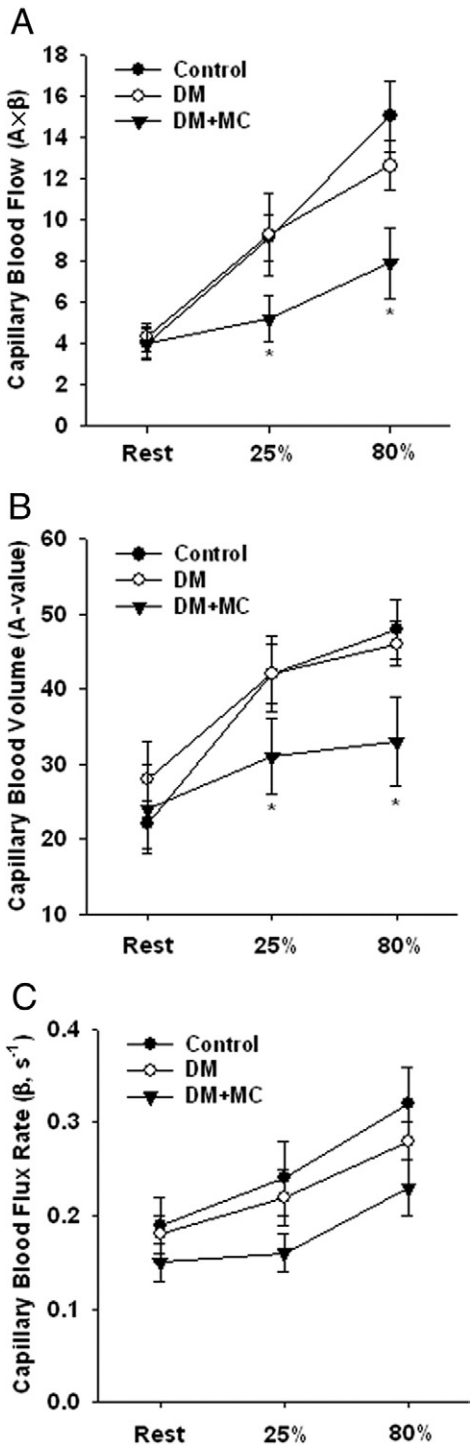


**Figure 5** Forearm Flexor Muscle Perfusion at Rest and During Exercise

**(A)** Capillary blood flow (A×$\beta$), **(B)** capillary blood volume (CBV) (A-value), and **(C)** capillary blood flux rate ($\beta$-value) in control subjects and in patients with either uncomplicated DM or DM + MC under resting conditions and during contractile exercise. The stepwise increase with incremental exercise level for all parameters was significant by the mixed-model analysis ($p < 0.001$ for flow, $p = 0.02$ for CBV, $p = 0.002$ for flux rate). *$p < 0.05$ (adjusted) versus control subjects. Abbreviations as in Figure 1.

ment in capillary response in patients with DM. Skeletal muscle capillary perfusion during exercise in diabetic states has become a topic of focus because of its influence on exercise tolerance and glucose metabolism. Exercise has been shown to augment insulin-mediated glucose uptake, at least partially through an increase in muscle flow (16,27). The ability to distinguish changes in CBV and capillary erythrocyte velocity during muscle contraction is important because of the uncoupling of limb arterial inflow and muscle microvascular perfusion during exercise (2). For example, in the current study, exercise-mediated increases in microvascular flow in control subjects were out of proportion to changes in brachial artery inflow. More importantly, despite similar brachial artery blood flow responses between the study groups, microvascular perfusion was found to be markedly abnormal in patients with DM + MC.

In patients with DM who did not have microvascular complications, capillary flow responses to exercise were essentially normal. This result was not entirely unexpected because skeletal muscle capillary blood flow responses to electrically stimulated contraction also are normal in Zucker diabetic fatty rats despite an impairment in insulin-mediated capillary recruitment (17). Differences in microvascular response to exercise and hyperinsulinemia are likely attributable to different regulatory mechanisms. Skeletal muscle flow during exercise is complex and involves multiple biochemical pathways and hydrodynamic forces that have been the subject of comprehensive reviews (28). Although NO is a requisite for normal insulin-mediated capillary recruitment, it does not participate in exercise-mediated microvascular recruitment and contributes only a minor role to exercise-mediated glucose uptake in a flow-independent fashion, possibly through transporter function (11,13).

Patients with microvascular complications of DM, all of whom had proteinuria as a qualifying criteria, responded to

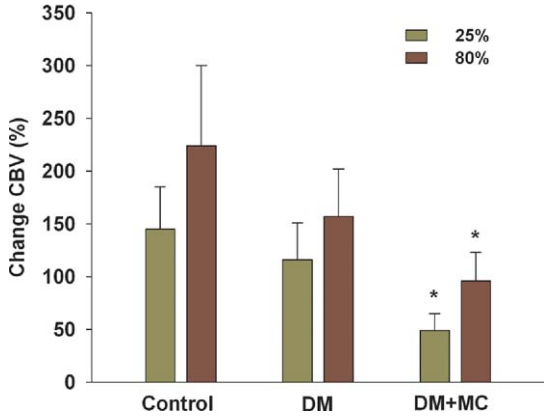


**Figure 6** Change in CBV During Exercise

Data shows percent change in CBV during 25% or 80% maximal grip force exercise. *$p < 0.05$ (adjusted) versus control subjects. Abbreviations as in Figures 1 and 5.

exercise in a different fashion than those with uncomplicated disease. In these patients capillary recruitment to contractile exercise was abnormal. The pathophysiologic basis for this finding is still undefined. We believe that a functional abnormality is more likely than morphologic capillary rarefaction since capillary recruitment was significantly impaired even at low-intensity (25%) contractile exercise before maximal CBV was reached. Although capillary responses were abnormal in the DM + MC group, brachial artery flow responses were normal. This finding implicates the distal microcirculation and possibly the inability to redistribute flow to muscle capillary beds from other limb tissues or non-nutritive pathways as the primary source of the defect.

The limited number of subjects did not allow for complex multivariate models to study all of the covariates that could possibly influence flow reserve. However, it was noted that patients with DM + MC were characterized by poorer control of plasma glucose and a greater degree of insulin resistance. Glucose levels correlated with blood viscosity, which was greatest in the DM + MC group. Blood viscosity is a critical determinant of flow at the capillary level and has been associated with the degree of insulin resistance and with diabetic complications (30–32), including proteinuria, which was a common criteria for all DM + MC patients in this trial (33). The pathophysiologic basis is probably multifactorial. The simple addition of D-glucose to blood or protein glycosylation does not appear to affect viscosity (34). Instead, it is more likely to the result of protein dysregulation associated with a heightened inflammatory state (35,36). This notion is supported by the relation we found between viscosity and CRP.

As an alternative hypothesis, elevated CRP may have contributed to abnormal capillary responses through its actions to decrease endothelial NO synthase activity (37). Although there was a correlation between viscosity and flow parameters during exercise, our model suggested that none of these variables added significant predictive information to group identity. These data suggest that differences in capillary response between groups could not be explained solely on the basis of these factors but also that statistical power was likely limited by study size.

**Study limitations.** The causal link between flow impairment and microvascular complications has not been proven and could probably only be achieved only by a large prospective study where the value of perfusion response is investigated in terms of predicting future complications. Defining the temporal relationship between perfusion abnormalities and complications is probably best suited to studies that use controlled animal models of disease. Exercise perfusion was assessed only under conditions of fasting. However, studies in which the authors used positron emission tomography have demonstrated that exercise-mediated augmentation in total blood flow is blunted in insulin-resistant subjects when exercise is performed under hyperinsulinemic conditions (16). We also did not estimate forearm muscle mass relative to other limb tissues, which may have influenced flow responses.

## Conclusions

We have demonstrated that capillary recruitment during low- and high-intensity exercise is impaired in patients with DM that have established microvascular complications of disease. Although our data suggest that rheologic abnormalities that are associated with the degree of insulin resistance may be responsible for these abnormalities, larger trials will be needed to control for the numerous clinical variables inherent in studying the diabetic population.

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**Key Words:** diabetes mellitus ■ contrast ultrasound ■ microvascular dysfunction ■ muscle perfusion ■ microbubbles.