Dynamics of microvascular oxygen pressure during rest-contraction transition in skeletal muscle of diabetic rats

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1Department of Kinesiology and Department of Anatomy and Physiology, Kansas State University, Manhattan, Kansas 66506-5802; 2Department of Medicine, University of California, La Jolla, California 92039-0623; and 3Department of Physiology, Kirksville College of Osteopathic Medicine, Kirksville, Missouri 63501

Received 23 January 2002; accepted in final form 7 May 2002

Behnke, Bradley J., Casey A. Kindig, Paul McDonough, David C. Poole, and William L. Sexton. Dynamics of microvascular oxygen pressure during rest-contraction transition in skeletal muscle of diabetic rats. Am J Physiol Heart Circ Physiol 283: H926–H932, 2002.—Type I diabetes reduces dramatically the capacity of skeletal muscle to receive oxygen (QO2). In control (C; n = 6) and streptozotocin-induced diabetic (D; n = 6), plasma glucose was 25.3 ± 3.9 mmol/l and C: 8.3 ± 0.5 mmol/l) rats, phosphorescence quenching was used to test the hypothesis that, in D rats, the decline in microvascular PO2 [PmO2, which reflects the dyquenching was used to test the hypothesis that, in D rats, the dyquenching was used to test the hypothesis that, in D rats, the
dynamic balance between O2 utilization (Vo2) and QO2 of the spinotrapezius muscle after the onset of electrical stimulation (1 Hz) would be faster compared with that of C rats. PmO2 data were fit with a one or two exponential process (contingent on the presence of an undershoot) with independent time delays using least-squares regression analysis. In D rats, PmO2, at rest was lower (C: 31.2 ± 3.2 mmHg; D: 24.3 ± 1.3 mmHg, P < 0.05) and at the onset of contractions decreased after a shorter delay (C: 13.5 ± 1.8 s; D: 7.6 ± 2.1 s, P < 0.05) and with a reduced mean response time (C: 31.4 ± 3.3 s; D: 23.9 ± 3.1 s, P < 0.05). PmO2 exhibited a marked undershoot of the end-stimulation response in D muscles (D: 3.3 ± 1.1 mmHg, P < 0.05), which was absent in C muscles. These results indicate an altered VO2-to-QO2 matching across the rest-exercise transition in muscles of D rats.

cellular perturbations of phosphocreatine, free ADP, and Pi, for example, that accelerate glycolgenolysis and utilization of finite glycogen reserves (33). This phenomenon is likely to contribute to the reduced exercise tolerance that is present in the diabetic condition. In healthy subjects, there is strong evidence that pulmonary VO2 kinetics closely reflect the dynamics of O2 exchange at the muscle (10). Moreover, it is known that streptozotocin-induced diabetes in rats elicits marked skeletal muscle structural (7, 19, 27), hemodynamic (8, 12, 19), and metabolic perturbations (6, 7). For instance, streptozotocin-induced diabetes causes marked muscle fiber atrophy (7, 19, 27), slows arteriolar vasodilation and capillary red blood cell (RBC) velocity (VRBC) (8, 12, 19), and reduces citrate synthase activity (19) and pyruvate-stimulated O2 consumption (6, 11).

Thus it appears that the ability to deliver O2 to muscle (QO2) as well as the capability of muscle to utilize that O2 (VO2) is impaired in diabetic rats. During electrical stimulation, phosphocreatine concentration falls to a greater extent in muscles of diabetic rats, and this is associated with a reduced ability to maintain submaximal force production during contractions (6). This finding is consistent with a lower oxidative enzyme capacity (15) and a reduced O2 availability to muscle mitochondria (13).

The technique of phosphorescence quenching measures PO2 at the site of blood-tissue O2 exchange (i.e., predominantly within the microcirculation, PmO2) and therefore provides information regarding the efficacy of the matching between muscle QO2 and VO2. We adapted this technique to monitor PmO2 in the rat spinotrapezius muscle at rest and across the transition to muscle contractions (3). The purpose of this study was to test the hypothesis that diabetes induces an impairment of the matching between muscle QO2 and VO2 such that PmO2 falls more rapidly and to a greater extent during the transition from rest-exercise than in healthy controls. Specifically, we hypothesized that in muscle of diabetic rats, after initiation of contractions, PmO2 would fall with little delay (the control response manifests a delay of 10–20 s) and would undershoot the steady-state level. If these responses are observed, they will provide evidence of a reduced ability of skel-
etal muscle in diabetes to adequately match $\dot{Q}_O_2$ and $V_O_2$ across the transition to an increased energetic requirement. Such information would support the notion that an impaired muscle $O_2$ delivery, concomitant with a decreased oxidative capacity, may be responsible for the slowed $V_O_2$ kinetics (large $O_2$ deficit) and thus contribute to the poor muscle performance in the diabetic population.

**METHODS**

**Experimental animals.** Female Sprague-Dawley rats ($n = 21$, initial weight $244 \pm 2$ g) were utilized in this investigation. The rats were randomly divided into control ($n = 9$) and diabetic ($n = 12$) groups. All surgical procedures and handling of the rats were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the protocols were approved by the Animal Care and Use Committee of Kansas State University. Animals assigned to the diabetic group received a single intracardiac injection of streptozotocin (50 mg/kg) in sterile citrate-buffered saline ($pH = 4.5$) while under halothane anesthesia (2% in 95% $O_2$-5% $CO_2$). The onset of diabetes was verified 48 h later using urine glucose test strips (Diastix; Ames, IA). All diabetic rats had a urine glucose concentration $>100$ mmol/l. Experiments were conducted 8 wk after confirmation of the diabetic state.

**Surgical preparation.** All surgical procedures were performed under pentobarbital anesthesia (50 mg/kg ip to effect and supplemented as necessary). The left carotid artery was cannulated (polyethylene-50, Intra-Medic polyethylene tubing, Clay Adams Brands; Sparks, MD) to monitor arterial blood pressure (model 200, DigiMed BPA; Louisville, KY) as well as to allow infusion of the phosphorescent probe and blood sampling.

The spinotrapezius muscle preparation is employed routinely for intravital microscopy studies of microvascular function. In the present investigation, a U-shaped skin incision exposed the left spinotrapezius muscle for phosphorescence measurements of $PmO_2$ and electrical stimulation. After resection of overlying skin and fascia, the muscle surface was superfused with Krebs-Henseleit solution equilibrated with 5% $CO_2$-95% $N_2$ at 38°C. Body temperature was maintained at 38°C using a heating pad. Stainless steel plate electrodes (2.5 mm diameter) were attached to each muscle proximal to the motor point (cathode) and across the caudal end (anode) close to the spinal attachment to elicit indirect, bipolar muscle contractions.

**Experimental protocol.** The phosphorescent probe R2 (30 mg/kg) was infused via the arterial cannula $\sim$10 min before each experiment. An arterial blood sample was taken for analysis of blood glucose concentration (Accu-Check Advantage, Boehringer-Mannheim), blood gases, $pH$ (Nova Stat Profile M; Waltham, MA), and pre- and postmeasurement hematocrit. Following a 10- to 15-min stabilization period after surgery, twitch muscle contractions were elicited at 1 Hz for 3 min (4–5 V, 2 ms pulse duration) using a Grass S88 stimulator (Quincy, MA). $PmO_2$ was determined at 2-s intervals at rest and after the rest-to-stimulation transition for 3 min. Mean arterial pressure (MAP) and body temperature were monitored continuously throughout the protocol. At the conclusion of each experiment, the rat was euthanized using an intra-arterial bolus of saturated KCl. The spinotrapezius muscle was dissected immediately and frozen for later analysis of citrate synthase activity.

**Microvascular $P_O_2$ measurements.** The oxygen dependence of the probe phosphorescence can be described quantitatively through the Stern-Volmer relationship (26). For the phosphorescent probe palladium meso-tetra(4-carboxyphenyl)porphyrin dendrimer (R2) bound to albumin at 33°C and $pH$ 7.4, the quenching constant is 409 Torr/s and lifetime of decay in absence of $O_2$ is 601 $\mu$s (20, 21). $PmO_2$ was determined using a PMOD 1000 Frequency Domain Phosphorometer (Oxygen Enterprises; Philadelphia, PA) with the common end of the bifurcated light guide placed $\sim$2–3 mm above the medial region of the spinotrapezius (i.e., superficial to dorsal surface). The PMOD 1000 uses a sinusoidal modulation of the excitation light (524 nm) at frequencies between 100 Hz and 20 kHz, which allows phosphorescence lifetime measurements from 10 $\mu$s to $\sim$2.5 ms. In the single frequency mode, 10 scans (100 ms) were used to acquire the resultant lifetime of the phosphorescence (700 nm) and repeated every 2 s (for a review, see Ref. 30). The phosphorescence lifetime was obtained by taking the logarithm of the intensity values at each time point and by fitting the linearized decay to a straight line by the least-squares method (4).

**Citrate synthase activity.** The citrate synthase activity within the spinotrapezius was determined spectrophotometrically at 30°C as described by Srere (28).

**Statistical analysis.** Curve fitting was accomplished using nonlinear regression (KaleidaGraph 3.5, Synergy software; Reading, PA) and was performed on the $PmO_2$ values using the following monoequational function

$$PmO_2(t) = PmO_2(b) - \Delta PmO_2(\delta t) \left[1 - e^{-t(\bar{\tau} - t)}\right]$$

and double-exponential function

$$PmO_2(t) = PmO_2(b) - \Delta PmO_2(\delta t) \left[1 - e^{-t(\bar{\tau} - t)}\right] + \Delta PmO_2(\delta t) \left[1 - e^{-t(\bar{\tau} - t)}\right]$$

where $PmO_2(t)$, $PmO_2(b)$ and $\Delta PmO_2(\delta t)$ are $PmO_2$ at time $t$, baseline (i.e., precontraction) $PmO_2$, and the decrease in $PmO_2$ from baseline to the end-stimulation values, respectively. For the more complex double-exponential model, $\Delta PmO_2(\delta t)$ designate the asymptotic value to which component of the $\Delta PmO_2$ is projecting, $\bar{\tau}_1$ and $\bar{\tau}_2$ are the time constants, and $T_D_1$ and $T_D_2$ are the independent time delays of the respective responses. Magnitude of the “undershoot” was assessed from the $PmO_2$ response independent of the curve-fitting model utilized. Goodness of fit was determined by three criteria: 1) the coefficient of determination (i.e., $r^2$), 2) the sum of the squared residuals, and 3) visual inspection and analysis of the residual fit to a linear model. Differences between baseline (precontraction), nadir, and end-stimulation $PmO_2$ were analyzed using a one-way repeated-measures ANOVA. When differences were detected by ANOVA, where these differences lie was determined using a Student-Newman-Keuls post hoc analysis. Differences between model parameter estimates [i.e., $\bar{\tau}_1$, $\bar{\tau}_2$, mean response time (MRT), etc.] were determined by an unpaired $t$-test. Significance was accepted at $P < 0.05$.

**RESULTS**

**Animals in which MAP fell below 60 mmHg and/or arterial $P_O_2$ fell below 80 mmHg (control: $n = 3$; diabetic: $n = 6$) were rejected, and their data were omitted from further analysis. By these criteria, successful experiments were completed in six control and six diabetic rats.**

**Animal data.** Blood glucose was significantly elevated and body weight and spinotrapezius muscle weight were less in the diabetic rats compared with...
control rats (Table 1). Spinotrapezius muscle weight-to-body weight ratio was lower in diabetic rats, reflecting marked muscle atrophy (Table 1). Diabetes resulted in a reduction in citrate synthase activity in the spinotrapezius muscle (Table 1). MAP during experiments, systemic hematocrit measured before (pre) and after (post) the experiments, pH, and arterial PO2 were not different in control and diabetic animals (Table 1).

**Spinotrapezius PmO2 response.** Baseline PmO2 in the resting state was lower in diabetic compared with control muscles (control: 31.2 ± 3.2 mmHg; diabetic: 24.3 ± 1.3 mmHg, P < 0.05; Table 2). The ΔPmO2 at end-stimulation (i.e., Δ1 + Δ2) was less in diabetic than control muscles (control: 15.7 ± 2.4 mmHg; diabetic: 8.8 ± 1.4 mmHg, P < 0.05). In addition, whereas the end-stimulation PmO2 values were not different between groups (control: 15.5 ± 2.4 mmHg; diabetic: 15.2 ± 2.3 mmHg), the PmO2 nadir was significantly lower in diabetic muscle (diabetic: 10.8 ± 1.6 mmHg; control: 15.5 ± 2.4 mmHg, P < 0.05). As depicted in Figs. 1 and 2, the profile of PmO2 at the onset of contractions was substantially different in control vs. diabetic muscles. Specifically, in spinotrapezius muscles of control rats, PmO2 remained at baseline levels by 10.2 ± 0.3 s on July 7, 2017 http://ajpheart.physiology.org/ Downloaded from

![Fig. 1. Dynamic microvascular PO2 (PmO2) profiles of the entire stimulation period for individual spinotrapezius muscles of control rats. Time 0 represents the start of 180 s of electrical stimulation.](image)

**Table 1. Animal data**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Diabetic (n = 6)</th>
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</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>284 ± 7</td>
<td>219 ± 11*</td>
</tr>
<tr>
<td>Spinotrapezius weight, mg</td>
<td>173 ± 17</td>
<td>100 ± 12*</td>
</tr>
<tr>
<td>Spinotrapezius-to-body weight ratio, mg/g</td>
<td>0.61 ± 0.06</td>
<td>0.48 ± 0.07†</td>
</tr>
<tr>
<td>Citrate synthase activity, μmol·min⁻¹·g⁻¹</td>
<td>15.2 ± 1.6</td>
<td>10.6 ± 1.0*</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>8.3 ± 0.5</td>
<td>25.3 ± 3.9*</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>101 ± 8</td>
<td>82 ± 7</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>49.0 ± 1.0</td>
<td>49.7 ± 1.6</td>
</tr>
<tr>
<td>Preexperiment</td>
<td>52.2 ± 0.7</td>
<td>48.2 ± 1.6</td>
</tr>
<tr>
<td>Postexperiment</td>
<td>89 ± 2</td>
<td>87 ± 7</td>
</tr>
<tr>
<td>Arterial PO2, mmHg</td>
<td>7.36 ± 0.01</td>
<td>7.34 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals. *P ≤ 0.05 compared with control; †P ≤ 0.05 compared with control (one-tailed test).

**Table 2. Characteristics of the PmO2 response in control and diabetic rats**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Diabetic (n = 6)</th>
</tr>
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<tbody>
<tr>
<td>Baseline PmO2, mmHg</td>
<td>31.2 ± 3.2</td>
<td>24.3 ± 1.3*</td>
</tr>
<tr>
<td>ΔPmO2 (rest-to-end-stimulation), mmHg</td>
<td>15.7 ± 2.4</td>
<td>8.8 ± 1.4*</td>
</tr>
<tr>
<td>End-stimulation PmO2, mmHg</td>
<td>15.5 ± 2.4</td>
<td>15.2 ± 2.3</td>
</tr>
<tr>
<td>TD1, s</td>
<td>13.5 ± 1.8</td>
<td>7.6 ± 2.1*</td>
</tr>
<tr>
<td>T1, s</td>
<td>17.9 ± 2.3</td>
<td>16.3 ± 2.7</td>
</tr>
<tr>
<td>T2, s</td>
<td>32.0 ± 3.3</td>
<td>22.8 ± 3.6†</td>
</tr>
<tr>
<td>MRT1, s</td>
<td>31.4 ± 3.3</td>
<td>23.9 ± 3.1†</td>
</tr>
<tr>
<td>TD2, s</td>
<td>NA</td>
<td>85.4 ± 18.6</td>
</tr>
<tr>
<td>T2, s</td>
<td>NA</td>
<td>85.4 ± 40.9</td>
</tr>
<tr>
<td>PmO2 undershoot, mmHg</td>
<td>0.5 ± 0.3</td>
<td>3.3 ± 1.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals. PmO2, microvascular PO2; TDi, initial time delay; T1, initial time constant; T2, time to achieve 63% of primary response (model independent); T2, second time delay; T2, second time constant; MRT1, mean response time of primary response (TD1 + T1); PmO2 undershoot, PmO2 end-stimulation − PmO2 minimum; NA, not applicable. *P ≤ 0.05 compared with control; †P ≤ 0.05 compared with control (one-tailed test).
control vs. diabetic muscles (Table 2). The MRT (i.e., $TD_1$) for the primary response was less in diabetic rats (control: 31.4 ± 3.3 s; diabetic: 23.9 ± 3.1 s, $P < 0.05$). The time necessary from the initiation of contractions for $PmO_2$ to fall to 63% of the difference between baseline and the nadir of the response ($T_{63}$), i.e., a model-independent estimate of the MRT, was also less in diabetic muscles (control: 32.0 ± 3.3 s; diabetic: 22.8 ± 3.6 s, $P < 0.05$).

For the control muscles, there was no evidence of a significant $PmO_2$ undershoot. In contrast, there was a consistent and marked $PmO_2$ undershoot in the diabetic muscles that ranged up to 7 mmHg (average ±3.5 mmHg; Table 2). In diabetic muscles, the double-exponential model identified a secondary increase in $PmO_2$ after a time delay ($TD_2$) of 85.4 ± 18.6 s with a $\tau_2$ of 85.4 ± 40.9 s (Fig. 2 and Table 2).

**DISCUSSION**

The results presented herein demonstrate that at rest and across the transition from rest to contractions, diabetes alters the dynamic relationship between $QO_2$ and $VO_2$. The decreased $PmO_2$ at rest is consistent with microvascular studies that have documented a reduced capillary RBC flux in muscles of diabetic rats (19). Moreover, across the rest-to-contractions transition, the accelerated fall of $PmO_2$ and subsequent undershoot are indicative of a comparatively sluggish $QO_2$ response. Thus both conductive ($QO_2$) and diffusive (driving pressure for blood-tissue $O_2$ exchange, $PmO_2$) elements of $O_2$ delivery and exchange are impaired in muscles of diabetic rats. The early reduction in $PmO_2$ would be expected to occur concomitant with a lower intramuscular $P_o2$, which has been linked mechanistically with slower $VO_2$ kinetics (9). Accordingly, the present findings offer a putative mechanistic basis for the slowed pulmonary $VO_2$ kinetics that is symptomatic of diabetic patients (2, 23).

**Phosphorescence quenching measurement of $P_o2$.** Oxygen is transported by bulk flow from the lungs to the skeletal muscle capillaries and by diffusion from the capillaries to the myocytes according to Fick’s Law. In the plasma, the content of oxygen is a function of pressure and solubility (which is very low, 0.003 ml $O_2$·100 ml$^{-1}$·mmHg$^{-1}$), such that the overall oxygen-carrying capacity of plasma is trivial compared with that of hemoglobin or myoglobin. The location of the plasma between these two principal $O_2$ carriers is such that plasma $P_o2$ (measured via phosphorescence quenching) (26) provides a sensitive indicator of the matching between muscle $VO_2$ (i.e., myocyte $O_2$ uptake) and $QO_2$. Therefore, as intracellular $P_o2$ falls as a result of increased mitochondrial $VO_2$, a greater extraction from the adjacent capillaries is expected based on Fick’s Law. It is these changes that are reflected by the prevailing $PmO_2$. As evident from Fick’s Law, for any given intramyocyte $P_o2$, a fall in $PmO_2$ will reduce the pressure gradient driving the diffusive movement of $O_2$ from the blood into the myocyte and, given a finite muscle $O_2$-diffusing capacity, will serve to limit blood-muscle $O_2$ transfer (i.e., $VO_2$) (14, 24, 31).
Diabetes model. Streptozotocin-induced diabetes leads to marked alterations in skeletal muscle morphology and hemodynamics that significantly impact the structural and functional capacity of the muscle to transport, exchange, and utilize O$_2$ at rest and during metabolic challenges (e.g., exercise). One of the most obvious changes induced by diabetes is a pronounced muscle fiber atrophy, which is associated with a decreased oxidative enzyme capacity (7, 19, 27) and maximal VO$_2$.

With respect to O$_2$ delivery within skeletal muscle, the morphometric and hemodynamic alterations that occur in diabetic skeletal muscle include 1) a decreased capillary-to-fiber ratio (27); 2) a decreased capillary luminal diameter (19, 27); 3) a reduced proportion of capillaries supporting continuous flow (19); and 4) a reduced capillary V$_{RBC}$ and RBC flux (F$_{RBC}$) (19). In addition, there is an enhanced RBC membrane protein glycosylation that serves to increase RBC rigidity (29, 32). This increased RBC rigidity and hence decreased deformability will reduce the ability of RBCs to pass freely into the narrow capillaries that are present in muscle from diabetic rats (19, 29). The net effect of these structural and functional deficits present in skeletal muscle of diabetic rats constrains oxygen delivery (as assessed by F$_{RBC}$) by up to 50% at rest (19). Although technical limitations have so far precluded determination of microvascular hemodynamics across the rest-contractions transition in the muscle of diabetic rats, it is probable that the RBC flow deficits manifested at rest (19) are present to some degree during contractions. Pertinent to this issue, Baron and colleagues (1) reported reduced skeletal muscle blood flows in Type 1 diabetes. In addition, diabetes has been shown to impair arteriolar vasoreactivity to both endothelium dependent (e.g., ACh) and independent (e.g., sodium nitroprusside) vasodilators (8, 12). One further consideration that may impact intramyocyte O$_2$ transport is the reduction in myoglobin content in the skeletal muscle of diabetic rats (17). Such a lowering of myoglobin is expected to decrease intramuscular O$_2$ stores and also impair facilitated diffusion of O$_2$ between the subsarcolemmal space and mitochondria.

Interpretation of data. At the onset of muscle contractions, ATP demand increases almost instantaneously. However, the time delay (TD) preceding the decrease of PmO$_2$ at the onset of muscle contractions need not imply that both VO$_2$ or QO$_2$ are stagnant, but rather that they increase in concert such that there is little or no change in the QO$_2$-to-VO$_2$ ratio and therefore PmO$_2$. Accordingly, the results of the present investigation (i.e., reduced TD before PmO$_2$ falling in diabetic muscle) demonstrate that across the rest-contractions transition, the duration for which the QO$_2$-to-VO$_2$ ratio is unaltered is attenuated in diabetic compared with control muscle (Figs. 1 and 2). The resultant overall faster kinetics (expressed by MRT and T$_{65}$) and undershoot in PmO$_2$ observed in the muscle of diabetic rats are consistent with an attenuated or slower muscle blood flow response (increased T$_{QO2}$) across the rest-contractions transition. In marked contrast to the control response, after a secondary delay (TD$_2$) that averaged 85 s, PmO$_2$ subsequently increased toward the end-stimulation level. Because a marked reduction of VO$_2$ at 80–90 s of contractions is highly unlikely, this response is consistent with the presence of a delayed rise in blood flow (and thus QO$_2$) in the diabetic muscle that is necessary to elevate the PmO$_2$ to a value commensurate with that found in control muscle at the end of stimulation. The suddenness of this response suggests an abrupt arterial vasodilatation at a time when the rate of VO$_2$ increase is relatively slow. It is also possible that, had the diabetic muscles been stimulated for >3 min, the PmO$_2$ may have risen above that seen for the control steady-state condition. Indeed, a reduced muscle O$_2$-diffusing capacity (as suggested from morphometric and intravital microscopy examination of diabetic muscle (19, 27)) would mandate an elevated PmO$_2$-to-intramyocyte P$_2$O$_2$ gradient at any given VO$_2$.

The altered regulation of QO$_2$-to-VO$_2$ ratio in diabetes at any given time point across the transition is also apparent through the increased periodicity and magnitude of oscillations in PmO$_2$, which is quantified by the $x^2$-values (diabetic: 61.7 ± 17.9; control: 27.8 ± 8.6, P < 0.05) of the model used (i.e., the magnitude of oscillations around the model fit). These data suggest that there is an inability to regulate and match QO$_2$ to VO$_2$ precisely within the microvasculature (i.e., as reflected in the oscillations around the model fit shown in Figs. 2 and 3) and is consistent with the reported impairment in myogenic reactivity of arterioles in diabetic rats (8, 12). In addition, the reduced deformability of RBCs in diabetic rats coupled with the decreased capillary luminal diameter (19, 27) would be expected to both reduce blood flow capacity and to alter the distribution of RBCs within the capillary bed of contracting muscle (19). Deficits in QO$_2$ in concert with reduced intramuscular myoglobin content (17) would further reduce the muscle O$_2$-buffering capacity and therefore shorten the TD that precedes the decrease in PmO$_2$, as observed in the present study. This rationale is strengthened by the presence of a decreased muscle oxidative capacity (7, 11, 19, 27), which would act to reduce the speed of the VO$_2$ response (for a review, see Ref. 22). That PmO$_2$ (i.e., QO$_2$-to-VO$_2$ ratio) falls more rapidly in diabetic muscle despite VO$_2$ dynamics that are expected to be slower strengthens our conclusion that the QO$_2$ response across the rest-contractions transition must be slowed. The direct consequence of impaired QO$_2$ and reduced PmO$_2$ across the transition is that blood-myocyte O$_2$ exchange will be reduced compared with that of the control in diabetic muscle. This condition will mandate exacerbation of intracellular perturbations (e.g., greater decrease in intracellular phosphocreatine, increase in free ADP, enhanced glycogenolysis) needed to support the energetic demand of the muscle (34). Such a rationale is consistent with the observations of Challiss et al. (6), who demonstrated a greater decrease in muscle phosphocreatine levels in diabetic rat muscle compared with control rats working at the same intensity.
Preparation considerations. The results presented herein must be considered within the context and limitations of the model used. For example, electrical stimulation recruits all muscle fiber types simultaneously in contrast to a physiological ordered recruitment pattern. In addition, the combination of the depressant effects of anesthesia on cardiovascular function and electrical muscle stimulation may slow the QO2 response compared with voluntary muscle contractions (3). Notwithstanding this consideration, it is evident that the control and diabetic rat muscles exhibited a profoundly different PmO2 profile with evidence for a QO2 limitation to O2 transfer in diabetic rat muscles but not in their control counterparts. It is pertinent that there was a tendency for MAP to be lower in the diabetic rats due possibly to their greater sensitivity to the anesthesia. However, correlation analyses revealed no significant relationship between MAP and either the PmO2 TD or the magnitude of the PmO2 undershoot found within the diabetic muscles. This analysis supports the notion that the differential pattern of the PmO2 response found in diabetic rat muscles was not induced secondary to any effects of anesthesia on MAP.

Relevance. Patients with Type 2 diabetes evidence slowed pulmonary VO2 kinetics at the onset of moderate intensity (i.e., sublactate threshold) exercise and a reduced maximum VO2 (23). The slowed VO2 kinetics arise from 1) a prolonged phase I (cardiodynamic phase) attributable to a sluggish cardiovascular response (16, 18, 23, 25); and 2) slowed phase II kinetics thought to reflect limitations in VO2 at the exercising muscle (2, 10). The present investigation provides the first empirical evidence that the dynamic relationship between muscle QO2 and VO2 is altered across the rest-contractions transition in diabetic muscle. Thus, consistent with a delayed increase of QO2 (relative to VO2), PmO2 falls more rapidly, and this effectively reduces the pressure gradient for blood-myocyte O2 diffusion. The presence of a PmO2 undershoot and secondary rise in diabetic but not control muscle indicates that QO2 may take substantially longer to reach end-stimulation levels in diabetic rat muscle. Thus the slowed pulmonary VO2 kinetics and increased O2 deficit incurred at exercise onset (5, 23) would be expected to arise from impaired conductive and diffusive O2 transport within skeletal muscle. Given the deterministic role of skeletal muscle in setting pulmonary VO2 kinetics and the data presented herein, it is quite possible that the training-induced speeding of pulmonary VO2 kinetics in diabetic patients demonstrated by Brandenburg et al. (5) is driven by improvements in microvascular function and O2 exchange and possibly increases in oxidative enzymes within the active skeletal muscle. However, this notion remains to be tested.

In conclusion, at the onset of muscle contractions, the PmO2 profile for diabetic rats differs profoundly from that observed in their healthy counterparts. Specifically, in diabetic rats, PmO2 falls faster and undershoots the end-stimulation response. This profile is indicative of a reduced QO2-to-VO2 ratio across the transition to contractions that must arise from comparatively sluggish QO2 kinetics, which impairs conductive O2 delivery and diffusive blood-myocyte O2 exchange within contracting muscle. This behavior at the microvascular level comitant with a reduced oxidative capacity offers a putative mechanistic explanation for the slowed pulmonary VO2 kinetics observed in diabetic patients that causes an increased O2 deficit and is associated with a reduced exercise tolerance.

We thank Troy E. Richardson and Janet K. Bailey for excellent technical support.

This work was funded in part by National Heart, Lung, and Blood Institute Grant HL-50306 (to D. C. Poole) and by the Heartland Affiliate of the American Heart Association (to W. L. Sexton).

REFERENCES


17. Iannuzzo CD, Lesser M, and Bautista F. Metabolic adaptations in skeletal muscle of streptozotocin-diabetic rats following


