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

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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 $QO_2$ and $VO_2$ 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 $VO_2$ 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 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 reflection 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 gasses, 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 PO$_2$ 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 35°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 monoeponential function

$$PmO_2(t) = PmO_2(b) - \Delta PmO_2(\omega(a)) \left[1 - e^{-\left(\text{TD1}\right)}\right]$$

and double-exponential function

$$PmO_2(t) = PmO_2(b) - \Delta PmO_2(\omega(\text{primary})) \left[1 - e^{-\left(\text{TD1}\right)}\right] + \Delta PmO_2(\omega(\text{secondary})) \left[1 - e^{-\left(\text{TD2}\right)}\right]$$

where $PmO_2(t)$, $PmO_2(b)$ and $\Delta PmO_2(\omega(a))$ 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, $\Delta PmO_2(\omega(\text{primary}))$ and $\Delta PmO_2(\omega(\text{secondary}))$ designate the asymptotic value to which that component of the $\Delta$PmO$_2$ is projecting. $\tau_1$ and $\tau_2$ are the time constants, and TD$_1$ and TD$_2$ 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 (precontracting), 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., TD, $\tau$, mean response time (MRT), etc.] were determined by an unplanned $t$-test. Significance was accepted at $P < 0.05$.

**RESULTS**

Animals in which MAP fell below 60 mmHg and/or arterial Po$_2$ 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., Δt1 + Δt2) 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 ± 2.7 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 for an average of ~14 s before decreasing monoexponentially to the end-stimulation value (Fig. 1). In contrast, in diabetic animals, PmO2 decreased either immediately at the onset of contractions or with a reduced delay (average about one-half of that seen in control muscles; Fig. 2, Table 2). Furthermore, in diabetic muscles, there was a consistent and significant undershoot (i.e., endcontracting minus absolute nadir value) of PmO2 (3.3 ± 1.1 mmHg), followed by a subsequent slow increase to a value at which PmO2 was not different between control and diabetic muscles (control: 15.5 ± 2.4 mmHg; diabetic: 15.2 ± 2.3 mmHg, *P* > 0.05) at 3 min of stimulation.

**Modeled PmO2 responses.** A simple monoexponential model fit the PmO2 responses observed in muscle of control rats well (*r*² = 0.985 ± 0.006, *χ*² = 27.8 ± 8.6; Fig. 1), and, consequently, a more complex model was not considered. As evident from Figs. 2 and 3, the more complex double-exponential model provided a substantially better fit to the PmO2 response in diabetic muscles, as confirmed by significantly lower *χ*²-error values (monoexponential: 129.9 ± 49.1; double exponential: 61.7 ± 17.9, *P* < 0.05).

Model parameters for control and diabetic muscles are presented in Table 2. The time delay (TD) associated with the start of the primary exponential decrease in PmO2 (TD1) was shorter in diabetic than in control muscles (Table 2). However, *t*1 was not different in

### Table 1. Animal data

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>284 ± 7</td>
<td>219 ± 11†</td>
</tr>
<tr>
<td>Spino trapezius 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 wet⁻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>49.2 ± 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>Arterial PO2, mmHg</td>
<td>89 ± 2</td>
<td>87 ± 7</td>
</tr>
<tr>
<td>pH</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</th>
<th>Diabetic</th>
</tr>
</thead>
<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 ± 1.1†</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; TD1, initial time delay; T1, initial time constant; T2, time to achieve 63% of primary response (model independent); TD2, 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., TD1/H11001/H9270 1) for the primary response was less in diabetic rats (control: 31.4/H11006 3.3 s; diabetic: 23.9/H11006 3.1 s, P/H11021 0.05). The time necessary from the initiation of contractions for PmO2 to fall to 63% of the difference between baseline and the nadir of the response (T63), i.e., a model-independent estimate of the MRT, was also less in diabetic muscles (control: 32.0/H11006 3.3 s; diabetic: 22.8/H11006 3.6 s, P/H11021 0.05).

For the control muscles, there was no evidence of a significant PmO2 undershoot. In contrast, there was a consistent and marked PmO2 undershoot in the diabetic muscles that ranged up to 7 mmHg (average/H18528 3.5 mmHg; Table 2). In diabetic muscles, the double-exponential model identified a secondary increase in PmO2 after a time delay (TD2) of 85.4 ± 18.6 s with a τ2 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 Q02 and VO2. The decreased PmO2 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 PmO2 and subsequent undershoot are indicative of a comparatively sluggish Q02 response. Thus both conductive (QO2) and diffusive (driving pressure for blood-tissue O2 exchange, PmO2) elements of O2 delivery and exchange are impaired in muscles of diabetic rats. The early reduction in PmO2 would be expected to occur concomitant with a lower intramuscular PO2, which has been linked mechanistically with slower VO2 kinetics (9). Accordingly, the present findings offer a putative mechanistic basis for the slowed pulmonary VO2 kinetics that is symptomatic of diabetic patients (2, 23).

Phosphorescence quenching measurement of PO2. 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 O2·100 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 O2 carriers is such that plasma PO2 (measured via phosphorescence quenching) (26) provides a sensitive indicator of the matching between muscle VO2 (i.e., myocyte O2 uptake) and QO2. Therefore, as intracellular PO2 falls as a result of increased mitochondrial VO2, a greater extraction from the adjacent capillaries is expected based on Fick’s Law. It is these changes that are reflected by the prevailing PmO2. As evident from Fick’s Law, for any given intramyocyte PO2, a fall in PmO2 will reduce the pressure gradient driving the diffusive movement of O2 from the blood into the myocyte and, given a finite muscle O2-diffusing capacity, will serve to limit blood-muscle O2 transfer (i.e., VO2) (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\textsubscript{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 V\textsubscript{O\textsubscript{2}}.

With respect to O\textsubscript{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\textsubscript{RBC} and RBC flux (F\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2} stores and also impair facilitated diffusion of O\textsubscript{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\textsubscript{1}) preceding the decrease of Pm\textsubscript{O\textsubscript{2}} at the onset of muscle contractions need not imply that both V\textsubscript{O\textsubscript{2}} or Q\textsubscript{O\textsubscript{2}} are stagnant, but rather that they increase in concert such that there is little or no change in the Q\textsubscript{O\textsubscript{2}}-to-V\textsubscript{O\textsubscript{2}} ratio and therefore Pm\textsubscript{O\textsubscript{2}}. Accordingly, the results of the present investigation (i.e., reduced TD before Pm\textsubscript{O\textsubscript{2}} falling in diabetic muscle) demonstrate that across the rest-contractions transition, the duration for which the Q\textsubscript{O\textsubscript{2}}-to-V\textsubscript{O\textsubscript{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\textsubscript{50}) and undershoot in Pm\textsubscript{O\textsubscript{2}} observed in the muscle of diabetic rats are consistent with an attenuated or slower muscle blood flow response (increased t\textsubscript{Q\textsubscript{O\textsubscript{2}}}) across the rest-contractions transition. In marked contrast to the control response, after a secondary delay (TD\textsubscript{2}) that averaged 85 s, Pm\textsubscript{O\textsubscript{2}} subsequently increased toward the end-stimulation level. Because a marked reduction of V\textsubscript{O\textsubscript{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 Q\textsubscript{O\textsubscript{2}}) in the diabetic muscle that is necessary to elevate the Pm\textsubscript{O\textsubscript{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 vasodilation at a time when the rate of V\textsubscript{O\textsubscript{2}} increase is relatively slow. It is also possible that, had the diabetic muscles been stimulated for >3 min, the Pm\textsubscript{O\textsubscript{2}} may have risen above that seen for the control steady-state condition. Indeed, a reduced muscle O\textsubscript{2}-diffusing capacity (as suggested from morphometric and intravital microscopy examination of diabetic muscle (19, 27)) would mandate an elevated Pm\textsubscript{O\textsubscript{2}}-to-intramyocyte PO\textsubscript{2} gradient at any given V\textsubscript{O\textsubscript{2}}.

The altered regulation of Q\textsubscript{O\textsubscript{2}}-to-V\textsubscript{O\textsubscript{2}} ratio in diabetes at any given time point across the transition is also apparent through the increased periodicity and magnitude of oscillations in Pm\textsubscript{O\textsubscript{2}}, which is quantified by the \chi\textsuperscript{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 Q\textsubscript{O\textsubscript{2}} to V\textsubscript{O\textsubscript{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 Q\textsubscript{O\textsubscript{2}} in concert with reduced intramuscular myoglobin content (17) would further reduce the muscle O\textsubscript{2}-buffering capacity and therefore shorten the TD that precedes the decrease in Pm\textsubscript{O\textsubscript{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 V\textsubscript{O\textsubscript{2}} response (for a review, see Ref. 22). That Pm\textsubscript{O\textsubscript{2}} (i.e., Q\textsubscript{O\textsubscript{2}}-to-V\textsubscript{O\textsubscript{2}} ratio) falls more rapidly in diabetic muscle despite V\textsubscript{O\textsubscript{2}} dynamics that are expected to be slower strengthens our conclusion that the Q\textsubscript{O\textsubscript{2}} response across the rest-contractions transition must be slowed. The direct consequence of impaired Q\textsubscript{O\textsubscript{2}} and reduced Pm\textsubscript{O\textsubscript{2}} across the transition is that blood-myocyte O\textsubscript{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 glycolgenolysis) 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 pressant 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 concomitant 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.

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REFERENCES


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