Effects of Type II diabetes on capillary hemodynamics in skeletal muscle

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This group of patients has also been shown to demonstrate higher amounts of Type IIb (highly glycolytic) muscle fibers by some (28) but not all (2) investigations.

Recently, preliminary work in our laboratory (37) has shown that muscle microvascular O2 partial pressures (PO2,mv) are reduced by ∼11 Torr at rest and during contractions in the mixed-fiber type spinotrapezius muscle of the Type II diabetic Goto-Kakizaki (GK) rats (i.e., PO2,mv in GK: ∼18 Torr vs. control: ∼29 Torr). This GK model has been considered to be highly representative of the Type II diabetic state in humans (16). The decreased PO2,mv found in the spinotrapezius muscle may be indicative of an impaired microvascular O2 delivery (QO2) relative to O2 utilization (VO2) (Refs. 6, 32). We reasoned that direct observation of the microcirculation may provide a mechanistic basis for this decreased PO2,mv found in GK rat muscle and offer putative insights into the exercise intolerance typical of diabetic humans (41).

Within skeletal muscle, a functional microvascular bed is necessary for the provision of an adequate supply of O2 and other nutrients, as well as for removal of metabolic waste products. The modeling studies of Federspiel and Popel (14) suggest that the number of red blood cells (RBCs) adjacent to a muscle fiber (i.e., capillary tube hematocrit multiplied by the length of capillaries) is critical for O2 exchange. The length of capillaries is highly representative of the Type II diabetic state in humans (16). The decreased PO2,mv found in the spinotrapezius muscle may be indicative of an impaired microvascular O2 delivery (QO2) relative to O2 utilization (VO2) (Refs. 6, 32). We reasoned that direct observation of the microcirculation may provide a mechanistic basis for this decreased PO2,mv found in GK rat muscle and offer putative insights into the exercise intolerance typical of diabetic humans (41).

Experimental animals. Healthy male Wistar rats (control; n = 5; body wt = 557 ± 19 g, 6–8 mo old) and age-matched male GK (Taconic Farm, Germantown, NY) spontaneously diabetic rats (n = 7; body wt = 426 ± 15 g) were used in this investigation. The GK rat

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is a nonobese, hyperglycemic, insulin-resistant rat strain that was developed by selectively breeding glucose-intolerant Wistar rats (i.e., ∼5 generations; Ref. 16). Furthermore, these rats have been reported to have similar or higher non-fasting insulin concentrations compared with age-matched control Wistar rats (36). Goto et al. (17) reported that GK rats require no specially formulated diet and healthy Wistar rats may serve as appropriate controls because they are of the same original strain as the GK rat.

All rats were kept in a controlled environment with a fixed 12:12-h light-dark cycle and with a room temperature maintained at ∼22°C. Both control and GK rats were provided conventional rodent chow and water ad libitum. All experimental conditions and surgical procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Surgical preparation. Animals were anesthetized with pentobarbital sodium (50 mg/kg ip to effect and supplemented as necessary). The rat was then placed on a heating pad (38°C) to maintain body temperature throughout the experimental protocol. To monitor arterial blood pressure and heart rate (model 200, Digimed BPA, Louisville, KY), the left carotid artery was cannulated (polyethylene-50, Intra-needle brand) and an arterial blood sample was taken from the same catheter during fasting (19). The right femoral artery was cannulated (polyethylene-50, Intra-needle brand) and a femoral arterial blood pressure and heart rate were monitored continuously (model 200, Digimed BPA, Louisville, KY). Blood pressure and heart rate (model 200, Digimed BPA, Louisville, KY) were recorded for 60 s of continuous, or 2) impeded flow or stopped flow for >10 s of 60 s. These criteria were further used for determination of percentage of flowing capillaries [i.e., (number of capillaries supporting RBC flow/total number of visible capillaries per area) × 100]. The presence and duration of RBC flow or the presence of stationary RBCs was also used to determine capillary lineal density (i.e., the number of capillaries per unit muscle width) and countercurrent flow. For all capillaries in which hemodynamics were assessed and where the capillary endothelium was clearly visible on both sides of the lumen, capillary luminal diameter (dL) was measured (2–4 measurements/capillary) with calipers accurate to ±0.25 mm (±0.17 μm at ×1,184 magnification).

Examination of the microvascular fields was conducted in real time and by frame-by-frame analysis techniques (30 frames/s). Sarcomere length was determined from sets of 10 consecutive in-register sarcomeres (i.e., distance between 11 consecutive A bands) measured parallel to the muscle fiber longitudinal axis. This measurement was repeated 3–4 times where sarcomeres were visible to obtain a mean sarcomere length for each viewing field. For each muscle fiber in which both sarcolemmal boundaries were visible on the screen, the apparent fiber width perpendicular to the longitudinal muscle fiber axis was measured at three locations, and a mean fiber width was determined for each fiber. RBC velocity (V_{RBC}) was determined in all capillaries that were continuously RBC perfused by following the RBC path length over several frames. RBC flux (F_{RBC}) was measured by counting the number of RBCs in a capillary passing an arbitrary point. For each capillary in which hemodynamic data were measured, capillary tube hematocrit (Ht_{cap}) was calculated by the following equation:

\[
Ht_{\text{cap}} = \frac{\text{volume}_{\text{RBC}} \times F_{\text{RBC}}}{\text{area} \times \pi \times (d/2)^2 \times V_{\text{RBC}}}
\]

where volume_{RBC} is RBC volume, which was taken to be 61 μm³ (Ref. 1), and capillaries were approximated as circular in cross section (from Ref. 13, as modified by Ref. 23).

Statistical analysis. All data are presented as means ± SE where the group mean is that of the individual muscles rather than individual capillary measurements across muscles. Differences between control and GK groups were tested with a Student’s t-test. Where there was clear precedence for an a priori directional hypothesis (i.e., decreased F_{RBC} and V_{RBC}), a one-tailed test was used. Statistical significance was accepted at the P < 0.05 level.

RESULTS

GK rats exhibited significantly higher fasting blood glucose levels compared with the healthy control rats (control: 105 ± 5 mg/dl; GK: 263 ± 34 mg/dl; P < 0.05). The GK rat is considered a nonobese model of Type II diabetes, and this was reflected in the average body weights (control: 557 ± 19 g; GK: 426 ± 15 g; P < 0.05). Spinotrapezius weights were also lower in the GK rat (control: 546 ± 25 mg; GK: 421 ± 14 mg; P < 0.05); however, when expressed as a ratio to body weight,
Table 1. Cardiovascular variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 5)</th>
<th>Diabetic (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>371 ± 13</td>
<td>437 ± 14*</td>
</tr>
<tr>
<td>Mean arterial pressure, Torr</td>
<td>117 ± 6</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>43 ± 1</td>
<td>42 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05

the GK and control groups did not differ (P > 0.05). Although sustained within a normal physiological range, heart rate was higher (P < 0.05; Table 1) in the GK rats, but MAP did not differ between the groups (P > 0.05; Table 1). Systemic (arterial blood) hematocrit, measured at the end of the experiment, did not differ between groups (control: 43 ± 1%; GK: 42 ± 1%; P > 0.05; Table 1).

Muscle structural data. Neither sarcomere length (control: 2.6 ± 0.1 μm; GK: 2.7 ± 0.1 μm; nor capillary diameter (control: 4.7 ± 0.1 μm; GK: 5.1 ± 0.2 μm) differed between groups (both P > 0.05). Although muscle fiber width was lower in the GK rats (control: 65.0 ± 5.2 μm; GK: 51.5 ± 3.9 μm; P < 0.05), total capillary lineal density (i.e., the total number of both perfused and non-RBC-perfused capillaries per unit fiber width) did not differ (P > 0.05) between the GK and control rats, but the lineal density of RBC flowing capillaries was attenuated in the GK rats (P < 0.05; Fig. 1).

Hemodynamic comparisons. There was a significant decrease in the percentage of RBC flowing capillaries in the GK rats compared with the control rats (control: 93 ± 3%; GK: 66 ± 5%) that occurred in the absence of any alterations in the incidence of countercurrent flowing capillaries (control: 24 ± 5%; GK: 19 ± 3%; P > 0.05). In the RBC-perfused capillaries of the individual muscles of GK rats, dramatic decreases were also found in Hct_cap (control: 33 ± 1%; GK: 23 ± 1%), V_RBC (control: 454 ± 20 μm/s; GK: 158 ± 26 μm/s), and F_RBC (control: 42 ± 4 RBCs/s; GK: 14 ± 3 RBCs/s). Furthermore, V_RBC and F_RBC were significantly correlated in the individual muscles (r² value = 0.928; P < 0.05; Fig. 2). Importantly, when the product of flowing lineal density and capillary diameter was calculated (i.e., an index of Q˙O₂), the blood flow per unit of muscle was markedly lower in the GK rat spinotrapezius compared with control (control: 813 ± 88 RBCs·s⁻¹·mm muscle⁻¹; GK: 227 ± 37 RBCs·s⁻¹·mm muscle⁻¹; P < 0.05; Fig. 3).

DISCUSSION

To the best of our knowledge, this is the first investigation to examine resting skeletal muscle capillary hemodynamics in Type II diabetes, and the data demonstrate that this disease impairs indexes of both perfusive (i.e., Q˙O₂) and diffusive O₂ conductance in the spinotrapezius muscle. Specifically, significant reductions were found in I) the proportion of continuously RBC-perfused capillaries (↓ 29%), 2) capillary V_RBC (↓ 65%), 3) capillary F_RBC (↓ 66%), and 4) Hct_cap (↓ 30%). From these data, an overall reduction in Q₀₂ close to 70% was calculated. These conclusions are consistent with preliminary findings of a reduced PO₂,mv in the spinotrapezius muscle of GK rats (37) and indicate the presence of a substantial impairment in muscle microcirculatory function in Type II diabetes.

Comparison with published literature. Spinotrapezius muscle fiber widths range from ~30 to 60 μm, with the smallest fibers noted in Type I diabetes (23) and chronic heart failure (44, 47) and the larger fibers (i.e., 60 μm) found within healthy muscles (23, 44, 47). Within the current study, muscle fiber widths and spinotrapezius weights were significantly smaller in
the GK rat versus the control rat, which may have been a potential consequence of the retarded growth reported within the GK rat strain (16). However, when expressed as a ratio of spinotrapezius weight to body weight, the groups did not differ.

In general, the hemodynamic measurements for the control rats in the present investigation are similar to those presented previously for the young healthy rat spinotrapezius (19, 21, 22, 23, 44, 47). This was true despite the body mass being greater in the present investigation (i.e., present, \( \sim 560 \) g; previous, 200–300 g). For example, the percentage of RBC-perfused capillaries (93%) found herein is encompassed within the 80–96% range established for this muscle. In contrast to the above, \( V_{RBC} \) and \( F_{RBC} \) were both somewhat higher than reported previously, and this may be attributed to some combination of increased age, body mass, and different breed. It is pertinent that Russell and colleagues (47) found an increased \( V_{RBC} \) and \( F_{RBC} \) in the spinotrapezius of aged (26–28 mo) Fischer \( \times \) Brown Norway hybrid rats versus their younger counterparts. In many respects, the capillary hemodynamics of the GK rats (\( \downarrow \% \)RBC-flowing capillaries, \( \downarrow V_{RBC} \), and \( \downarrow F_{RBC} \)) resemble those found in rats suffering from Type I diabetes (23) or chronic heart failure (21, 44) with the magnitude of these perturbations large enough to discriminate the Type II diabetic rat from any of the healthy populations examined in the studies cited above. One exception to this statement was the \( Hct_{cap} \) in the GK animals (23 \( \pm \) 1%), which fell within the range established for healthy rats (e.g., Refs. 19, 47) but which was significantly lower than that found in the healthy control rats in the present investigation (33 \( \pm \) 1%).

**Theoretical basis for hemodynamic alterations.** In the present investigation, great care was taken to ensure that the muscles were not stretched to sarcomere lengths that cause a stretch-induced reduction of the capillary luminal diameter and active arteriolar vasoconstriction, both of which would be expected to decrease \( V_{RBC} \) and \( F_{RBC} \) (22, 39, 40, 52). In contrast, the following mechanisms might be implicated in the hemodynamic impairments found in the GK rats. 1) Increased glycosylation of the RBC membrane protein associated with Type II diabetes would increase RBC rigidity and blood viscosity (9, 27, 33), thereby limiting the ability of RBCs to travel freely through the capillary bed. 2) Acute hyperglycemia (but not chronic) has been reported to adversely affect the microvasculature by altering endothelial glycoalyx function and decreasing functional capillary density (55). This suggests that, preceding the diabetic condition, hyperglycemia compromises endothelial and smooth muscle function and may reduce RBC flux and velocity at the capillary level. 3) Because blood flow to the capillary is primarily controlled at the arteriolar level (Refs. 12, 38), it is quite plausible that the reduced number of RBC-flowing capillaries and slowed capillary hemodynamics may be the result of impaired vasomotor control. The GK rat model does demonstrate a high degree of arteriolar tone consequent to impaired endothelium-dependent vasodilation (7). This phenomenon also occurs in human diabetic patients (15, 33, 49, 51, 54). 4) Similar to that observed in humans (29, 35), GK rats demonstrate increased plasma concentrations of the potent vasoconstrictor endothelin-1 (5). In human diabetic patients, decreased vascular endothelial function has been implicated in the reduced basal forearm blood flow (42, 53) and leg blood flow (24) at rest and during muscle contractions. Thus, from this evidence, there is support for the notion that vascular dysfunction (endothelial and smooth muscle) in the GK rat may be responsible for the impaired capillary hemodynamics reported in the present investigation. There was certainly no evidence of a reduced capillary luminal diameter (as found in Type I diabetes; Ref. 23) or any structural impediments within the non-RBC-flowing capillaries.

**Implications of impaired hemodynamics.** According to the elegant modeling studies of Federispens and Popel (14) and Grobe and Thews (18), the effective \( O_2 \) diffusing capacity of muscle (\( D_{O_2,m} \)) is dependent on capillary tube hematocrit (i.e., the number of RBCs contained per unit length of capillary) and the length of RBC-perfused capillaries adjacent to the muscle fibers, as these indexes determine the number of RBCs available for blood-myoocyte \( O_2 \) transfer at any given instant. Fractional \( O_2 \) extraction and thus \( P_{O_2,mv} \), which constitutes the driving pressure facilitating blood-myoocyte \( O_2 \) movement, are determined by the relationship between \( D_{O_2,m} \) and blood flow (Q) such that \( V_{O_2} = QO_2(1 - e^{-D_{O_2,m}\beta Q}) \) and therefore \( V_{O_2}/Q_{O_2} = O_2 \) extraction = \( 1 - e^{-D_{O_2,m}\beta Q} \), where \( \beta \) is the slope of the \( O_2 \) dissociation curve in the physiologically relevant range (46, 50). In the present investigation, we found that \( Hct_{cap} \) was significantly decreased in diabetic muscle along with a modest reduction in the linear density of RBC-perfused capillaries, indicating that \( D_{O_2,m} \) is expected to be \( \sim 40\% \) lower in the GK rat spinotrapezius. With respect to \( Q_{O_2} \), this index of perfusive \( O_2 \) delivery fell \( \sim 70\% \) such that the critical ratio \( D_{O_2}/\beta Q \) and thus \( O_2 \) extraction will actually be higher in the GK muscle microcirculation. This finding provides a mechanistic basis for the lowered \( P_{O_2,mv} \) (37) and greater perturbation of the energetic state (i.e., \( \Delta[ADP]_{free} \), \( \Delta[phosphocreatine] \); Ref. 8) observed in muscle of individuals suffering from Type II diabetes.

The impaired capillary hemodynamics evidenced in the Type II diabetic GK rat herein help to explain the reduced \( P_{O_2,mv} \) (i.e., \( \sim 11 \) Torr) observed recently in this preparation (37) and are also consistent with the reduced limb or muscle blood flows reported in diabetic humans (24, 42, 53). However, at first glance, it is difficult to reconcile a lowered intramuscular \( P_{O_2,mv} \) (i.e., increased fractional \( O_2 \) extraction) with the reduced whole body fractional \( O_2 \) extraction reported by Baldi and colleagues (4). One putative explanation is that there is a mismatching of \( Q_{O_2} \) and \( V_{O_2} \) within and/or among muscles such that there are regions of under- and overperfusion. Akin to ventilation-perfusion mismatching in the lung (but directionally opposite), the slope of the \( O_2 \) dissociation curve at the low \( P_{O_2} \) will dictate that such mismatching acts to decrease overall \( O_2 \) extraction and increases venous \( P_{O_2} \). Specifically, in regions of the very flat portion of the \( O_2 \) dissociation curve at low \( P_{O_2} \), the slope of the curve is flattest, and there is a greater mismatch of \( V_{O_2} \) and \( Q_{O_2} \) (as found in Type I diabetes; Ref. 23) or any structural impediments within the non-RBC-flowing capillaries.
The consequences of a reduced $P_{O_2, mv}$ in the diabetic resting muscle may assume greater significance during exercise when greater $V_O_2$ are required to support cellular energetics. To achieve a given $V_O_2$ while exercising under conditions of reduced or impaired $Q_{O_2}$, diabetic muscle would have to increment fractional $O_2$ extraction further and exacerbate the decrease in intracellular $P_{O_2}$ to a level below that found in healthy muscle. As stated above, this situation will reduce the energy state of the myocytes, and this will result in enhanced utilization of glucose, glycogen degradation, and exacerbation of intracellular acid-base disturbances. These events are even more unfavorable when pathological components of a disease such as Type II diabetes includes mitochondrial dysfunction (20, 26) along with impaired glucose uptake and regulation.

Methodological considerations. When interpreting the results of intravital microscopy observations, three concerns are paramount. 1) The exteriorization procedure itself must not impact the measurements themselves. We have demonstrated that neither the spinotrapezius blood flow nor microvascular oxygenation is altered significantly by the surgical interventions necessary to view the microcirculation (3). 2) Image clarity may obscure key structures and bias measurements. It is true that small, non-RBC-perfused capillaries may not be readily discernable. However, Damon and Duling (10) reported that only ~2% of capillaries fell into this category, and those that did would be ineffective for delivering $O_2$ to the tissue. Notwithstanding this latter point, total capillary density was not different between control and GK muscles, and the principal differences between groups were found in the proportion of RBC-perfused capillaries and their dynamics. 3) Only a relatively small area ($270 \times 210 \mu$m) of tissue per screen was available for observation. Accordingly, the analyses were conducted on five different areas per muscle, and these were not significantly different from one another with respect to the measurements of interest. This finding suggests that the analysis did provide an adequate representation of the microcirculatory hemodynamics occurring in the control and GK spinotrapezius muscles.

In conclusion, the present investigation demonstrates that, within the spinotrapezius muscle of Type II diabetic rats, there is a significant attenuation in the percentage of capillaries supporting RBC perfusion. Moreover, within flowing capillaries, RBC hemodynamics are impaired ($\downarrow V_{RBC}$ and $\downarrow F_{RBC}$), and Hct$_{cap}$ is reduced. These changes occur in the absence of marked structural alterations. If these effects are present during muscle contractions, they may contribute to the $O_2$ exchange impairment and exercise intolerance characteristic of Type II diabetic patients. Irrespective of this, the GK rat model of Type II diabetes appears to offer a unique window through which to investigate the microcirculatory perturbations that accompany this all-too-prevalent disease. Specifically, the GK rat may prove invaluable for developing and determining the efficacy of different therapeutic modalities designed to limit or reverse the microcirculatory consequences of this disease. One key “next” experiment would be to establish whether correction of blood glucose can reverse the microcirculatory dysfunction established herein.

**REFERENCES**


**GRANTS**

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