Spinotrapezius muscle microcirculatory function: effects of surgical exteriorization

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The viability of skeletal muscle depends on the presence of a functional microvascular bed that provides adequate supply of oxygen and nutrients to, and removal of waste products from, the tissue. Important insights into muscle microcirculatory function in health and disease have been achieved with the use of intravital microscopy techniques that necessitate surgical exteriorization of the muscle. In this regard, the rat spinotrapezius preparation first described by Gray in 1973 (9) represents one principal model in which to evaluate physiological and pathophysiological phenomena within the microcirculation. For example, the rat spinotrapezius has been pivotal in our understanding of the effects of muscle structure-function relationships and smooth muscle physiology (16, 19, 33) and the role of nitric oxide and calcium in the microcirculation in health (24, 41). In addition, the spinotrapezius muscle preparation has provided insights into the pathophysiological microcirculatory consequences of chronic diseases such as type-1 diabetes (14, 35), hypertension (30), and chronic heart failure (6, 15). The tacit assumption in all of those studies has been that surgical intervention does not impact microcirculatory function either under control conditions or in response to experimental or pathological stimuli. However, it has been demonstrated that arterial and arteriolar pressures are reduced in the cremaster muscle after an exteriorization procedure that interrupts the distal blood supply emanating from the deferential artery (5).

To evaluate the effect of muscle exteriorization on the functional integrity of the microcirculation, the experimental methodology employed must be suitable for evaluation of not only blood flow (O2 delivery) but also the balance between O2 delivery and utilization in hypoxia; hyperoxia; metabolic blockade; muscle contractions; phosphorescence quenching; intravital microscopy.
both the intact and exteriorized preparations. Moreover, in addition to steady-state conditions, the dynamic response of the microcirculation should be evaluated. Consequently, a novel combination of phosphorescence quenching and microscope techniques was utilized across a range of different experimental conditions, i.e., metabolic stimulation with 2,4-dinitrophenol (DNP), electrically stimulated muscle contractions, and inspired hypoxia and hyperoxia, designed to evaluate the effect of surgical intervention on integrated static and dynamic microcirculatory behaviors within the rat spinotrapezius muscle.

**METHODS**

**Animals.** We used 4- to 5-mo-old female Sprague-Dawley rats ($n = 33, 215–265$ g) in these investigations. At this body mass, the spinotrapezius muscle has minimal overlying fascia and thus maintains optimal optical clarity for intravital microscopy.

**Spinotrapezius model.** The rat spinotrapezius muscle lies in the mid-dorsal region, where it originates in the lower thoracic and upper lumbar region and inserts onto the spine of the scapula. The muscle was prepared surgically with the rat under pentobarbital sodium anesthesia (40 mg/kg ip) with the use of previously described methods (9, 33). Exteriorization was always performed on the left (L) muscle. Although removal of the fascia does improve visibility of the fibers and microcirculation, it causes separation of the fibers and dilatation of microvessels (22, 27). Therefore, exteriorization was performed with minimal fascial disturbance designed to minimize tissue damage (i.e., with the exception of the distal feed artery, all of the vascular and nervous connections remained intact) and alterations of myocyte-to- vessel orientation (22) as well as any associated microcirculatory consequences (9, 27). Briefly, the rat was positioned on a circulation-heated (38°C) Lucite platform, and the spinotrapezius muscle was superfused continuously with the use of a microcirculator (model U3–7A, Julabo, Schwarzwald, Germany) with a Krebs-Henseleit bicarbonate-buffered solution equilibrated with 95% N$_2$-5% CO$_2$ (42). The exposed dorsal surface of the spinotrapezius muscle was protected with Saran Wrap (Dow Chemical, Indianapolis, IN), whereas the muscle was sutured with 6.0 silk at five equidistant positions around the caudal periphery to a thin wire horseshoe mani- fold (40). Thereafter, all exposed surfaces were either covered with Saran Wrap or bathed in the Krebs-Henseleit solution to $\sim 1$ mm in depth. The manifold was attached with a swirl to a muscle-stretching apparatus that permitted precise, systematic, and uniform length changes of the entire spinotrapezius muscle along its principal fiber longitudinal axis. This facilitates setting muscle sarcomere length at 2.7 $\mu$m, the approximate resting length, thus controlling for length-induced blood flow alterations (33).

To verify that the preparation was not contaminated by atmospheric O$_2$ diffusing through the fluid and Saran Wrap barriers, the microvascular PO$_2$ was monitored after the rat was euthanized (0.3 ml, 1 M KCl ia). In each instance, PO$_2$ fell rapidly to <1 mmHg, indicative of negligible diffusion of atmospheric O$_2$ into the muscle.

In those preparations where mean arterial pressure (MAP) fell $<90$ mmHg or there was bleeding visible in the vicinity of the spinotrapezius muscle, i.e., from damage to a feed vessel, the preparation was discarded ($\sim 30\%$). For the intact preparation, the surface of the contralateral, or right (R), muscle was exposed with a $\sim 2.5$-cm incision that facilitated superfusion, electrical stimulation, and measurement of microvascular PO$_2$.

**Experimental design and conditions.** The rats were assigned at random to one of four groups to determine the effects of surgical exteriorization on the spinotrapezius. Group 1 consisted of blood flow measurements under control conditions. In these studies, blood flow was measured in the anesthetized animal, and the L spinotrapezius muscle was then exteriorized and the R muscle exposed before a second blood flow measurement ($n = 4$) was made. In group 2, the blood flow response to increases in muscular metabolic activity induced by metabolic blockade was measured. Blood flow was measured after exposure in the intact R muscle and exteriorization in the L muscle, and both muscles were then superfused with 30 mM DNP before the second blood flow measurement ($n = 6$). Group 3 determined the response of microvascular PO$_2$ to electrical stimulation-induced muscle contractions (see **Electrical stimulation**). Intact and exteriorized muscles were stimulated in random order, and microvascular PO$_2$ was measured at rest and after 2–3 min stimulation when the microvascular PO$_2$ had stabilized ($n = 6$). In group 4, the dynamic response of microvascular PO$_2$ to hypoxic (100%) and hypoxic (10%) switches of inspired O$_2$ was measured. Measurements of microvascular PO$_2$ were made in intact and exteriorized muscles in random order across the transitions to altered inspired O$_2$ ($n = 7$). During each of the experimental conditions, MAP was monitored via the carotid artery (model 200, DigiMed BPA, Louisville, KY).

**Blood flow.** Tissue blood flows were determined with the use of the radionuclide-tagged microsphere technique that has been adapted for use in the exercising rat as described originally by Armstrong and Laughlin (1) and modified for use in our laboratories (29). Briefly, polyethylene catheters (PE-10 connected to PE-50) were placed into the right carotid artery and caudal artery. The right carotid artery catheter was advanced toward the heart and secured in a position just inside the aortic arch. This was accomplished by advancing the catheter toward the left ventricle while the arterial pressure waveform was being monitored. When the catheter reached the aortic valve, the pressure waveform became distorted. The catheter was retracted $2–3$ mm and secured in place. The caudal artery catheter was advanced toward the bifurcation of the descending aorta and secured in place. The right carotid artery catheter was connected to a pressure transducer, and the caudal artery catheter was connected to a 1-ml syringe placed in a withdrawal pump (model 907, Harvard Apparatus, South Natick, MA). Blood withdrawal from the tail artery catheter (microspheres reference sample) was initiated at a rate of 0.25 ml/min. At the same time, arterial blood pressure and heart rate (HR) were recorded from the carotid artery catheter. After 30 s of blood withdrawal, the carotid artery catheter was disconnected from the pressure transducer, and arterial blood pressure and heart rate were recorded from the carotid artery catheter. After 30 s of blood withdrawal, the carotid artery catheter was disconnected from the pressure transducer, and $\sim 250,000$ radioactive microspheres were slowly infused into the aortic arch. The microspheres ($^{46}$Sc, $^{85}$Sr, and $^{113}$Sn) used in the present study were $15 \pm 3$ $\mu$m in diameter as specified by the manufacturer (NEN Research Products, DuPont, Boston, MA). These isotopes were infused in random order under the different experimental conditions in which blood flows were measured (i.e., presurgery, postexperi- mentation, and post-DNP superfusion). The microspheres were suspended in normal saline containing 0.01% Tween 80 with a specific activity ranging from 7 to 15 mCi/g. Before each infusion, the microspheres were thoroughly mixed and agitated by sonication to prevent clumping. The micro- spheres were injected into the ascending aorta in a volume of $\sim 0.10$ ml over 5–10 s. Blood withdrawal from the caudal artery catheter was maintained for 30 s after the micro-
sphere injection to ensure that all microspheres had been cleared from the withdrawal catheter. The radioactivity of the tissues was determined on a Cobra II Auto-Gamma Spectrometer (Packard Instruments, Downers Grove, IL) set to record the peak energy activity of each isotope for 2 min. The radioactivity of the tissues was then analyzed by computer, taking into account the cross-talk fraction between the different isotopes. Blood flow (expressed as ml·min⁻¹·100 g⁻¹ of tissue) to the right and left spinotrapezius muscles, solei, and kidneys was calculated by the reference sample method as described by Ishise et al. (13). Adequate mixing of the microspheres was verified for each injection by demonstrating <15% difference in blood flows to the right and left kidneys and/or solei.

Phosphorescence quenching theory. The oxygen dependence of phosphorescence is described by the Stern-Volmer equation (37)

\[ \frac{t_0}{t} = 1 + k_q \times t_0 \times \frac{P_{O_2}}{k_q} \]

where \( t_0 \) and \( t \) are the phosphorescence lifetimes in the absence of oxygen and at an oxygen pressure microvascular \( P_{O_2} \). The quenching constant (\( k_q \)) is a second-order rate constant related to the frequency of collisions between \( O_2 \) and the excited triplet state of the porphyrin and the probability of energy transfer when collisions occur. \( P_{O_2} \) is calculated as

\[ P_{O_2} (\text{mmHg}) = \left( \frac{t_0}{t} - 1 \right) \left( k_q \times t_0 \times 10^{-6} \right)^{-1} \]

where \( t_0 \) and \( t \) are expressed in microseconds and \( k_q \) in mmHg⁻¹·s⁻¹. At 38°C and pH 7.4, \( k_q = 409 \text{ mmHg}^{-1} \cdot \text{s}^{-1} \) and \( t_0 = 601 \mu\text{s} \) (26).

Measurement. Initially, 15 mg/kg of the phosphorescent probe palladium-mesotetra(4-carboxyphenyl) porphyrin dimer (R2) was infused via arterial cannula. Oxyphor R2 binds tightly to albumin. Specifically, Lo et al. (25) have demonstrated that R2 is essentially completely bound to albumin in solution by a concentration of 0.5% albumin. The concentration of albumin in rat serum is approximately 6-fold that necessary for complete binding; see Ref. 36. In addition, R2 at a pH of 7.4 possesses a net-negative charge of approximately −14 mV. Both of these properties help to restrict R2 to the intravascular compartment. Microvascular \( P_{O_2} \) was determined by using a PMOD 1000 Frequency Domain Phosphorimeter (Oxygen Enterprises, Philadelphia, PA) with the common end of the bifurcated light guide placed ~2–4 mm above the medial region of the spinotrapezius (i.e., superficial to dorsal surface). This location on the spinotrapezius muscle is the same as that used during intravital microscopy and is ~8–10 mm from the distal (detached) proximity. The excitation light (524 nm) is focused on an ~2-mm diameter circle of exposed or exteriorized muscle surface and samples blood within the microvasculature up to 500 μm deep. The value of microvascular \( P_{O_2} \) principally reflects capillary blood, because this compartment constitutes the majority of intramuscular blood volume (34). The phosphorescence signal (700 nm) was averaged for a 200-ms interval for each microvascular \( P_{O_2} \) measurement, and the measurements were repeated at 2-s intervals.

Electrical stimulation. Stainless steel plate electrodes (2.5-mm diameter) were attached to the muscle proximal to the motor point (cathode) and across the caudal end (anode) close to the spinal attachment to elicit indirect bipolar muscle contractions (31). The muscle was stimulated to contract at 1 Hz for 3 min (5–8 V, 250-μs pulse duration) with the use of a stimulator (model S88, Grass Instruments, Quincy, MA). This stimulation protocol has been demonstrated to increase blood flow two- to threefold within the spinotrapezius muscle (Behnke, Kindig, Musch, and Poole; unpublished results).

Statistical analyses. Data are presented as means ± SE. The differences pre- and postsurgery and between intact and exteriorized muscles under each experimental condition were evaluated with the use of a two-way ANOVA with repeated measures design and paired t-tests as appropriate. Statistical significance was accepted at the \( P \leq 0.05 \) level.

RESULTS

Groups 1 and 2: Surgical exteriorization and DNP effects on blood flow. Surgical preparation did not alter whole muscle blood flow significantly within either the exteriorized or intact muscles (Fig. 1). Metabolic stimulation with DNP superfusion elevated muscle blood flow over twofold in the intact (19 ± 7 to 42 ± 14 ml·min⁻¹·100 g⁻¹, \( P < 0.05 \)) and exteriorized (16 ± 4 to 35 ± 9 ml·min⁻¹·100 g⁻¹, \( P < 0.05 \)) muscles. The magnitude of this increase was not different between intact and exteriorized muscles (Fig. 2).

Group 3: Effects of electrically stimulated muscle contractions on microvascular \( P_{O_2} \). Muscle contractions reduced microvascular \( P_{O_2} \) from 30.4 ± 4.3 to 21.8 ± 4.8 mmHg in intact and from 33.2 ± 3.0 to 25.9 ± 2.8 mmHg in exteriorized muscles (Fig. 3). Both conditions showed no difference in microvascular \( P_{O_2} \) between intact and exteriorized muscles.

Group 4: Effects of hypoxic and hyperoxic switches on microvascular \( P_{O_2} \). As expected, arterial \( P_{O_2} \) was significantly reduced from 80 ± 3 mmHg breathing room air to 48 ± 5 mmHg on the hypoxic (10% \( O_2 \)) inspirate. Inspired hyperoxia (100% \( O_2 \)) elevated arterial \( P_{O_2} \) to 307 ± 57 mmHg. Compared with MAP in normoxia (108 ± 4 mmHg), hypoxia significantly lowered MAP to 96 ± 4 mmHg (\( P < 0.05 \)), whereas it was unchanged in hyperoxia (110 ± 5 mmHg). Figure 4 demonstrates the magnitude and time course of the microvascular \( P_{O_2} \).
changes in response to switches among these inspired O2 percentages in one exteriorized muscle. As seen in Table 1, neither the magnitude nor the half-time dynamics of the microvascular PO2 response after each switch were altered significantly in exteriorized compared with intact muscles.

DISCUSSION

The principal methods for assessing the viability of an exteriorized muscle for microcirculatory investigation include the appearance of vigorous arteriolar flow and arteriolar vasodilation or vasoconstriction during chemical (e.g., adenosine, epinephrine) or oxygen (hypoxic, hyperoxic) challenge. Such assessment may be subjective and is almost never quantitative. Moreover, the inability to observe anything but superficial microcirculatory flow (reflectance microscopy) in the nonexteriorized preparation complicates direct observation of arteriolar vasoaction in intact muscle and thus comparison between intact and exteriorized preparations is not feasible. Thus the present investigation utilized methods and conditions suitable for assessing integrated microcirculatory function in both intact and exteriorized preparations. In no instance was there any indication of a systematic alteration in the basal blood flow (whole muscle) or microvascular PO2 or the magnitude and/or kinetic response of these variables to metabolic stimulation and change of muscle influent PO2 postsurgery. The present investigation therefore provides no evidence that the surgical manipulations requisite for exteriorizing the spinotrapezius before intravital transmission microscopy systematically impair microvascular integrity or responses across the range of conditions evaluated herein.

Surgical exteriorization. Skeletal muscle oxygen consumption (Vo2) increases up to two orders of magnitude from rest to maximal exercise (7, 20, 21, 32). This extraordinary increase necessitates a substantial elevation of muscle O2-diffusing capacity in concert with elevated convective O2 delivery. Theoretical models of O2 transport and diffusion within muscle consider that one major determinant of muscle O2-diffusing capacity is the number of red blood cells (RBC) found along the surface of a myocyte at a given time (8, 10). Thus at first it seems reasonable that at exercise onset more capillaries are recruited and the hematocrit within

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**Fig. 2.** R intact (I) exposed and L (exteriorized, EX) spinotrapezius muscle blood flow under baseline conditions (left) and during superfusion with 30 mM 2,4-dinitrophenol (DNP) (right). All values are means ± SE, n = 6. *P < 0.05, significant increase from baseline to DNP for R and L muscles.

**Fig. 3.** Spinotrapezius microvascular PO2 (PO2m) at rest (baseline, left) and in the steady state at the end of 2–3 min of 1-Hz contractions (right). All values are means ± SE, n = 6. *P < 0.05, significant decrease in PO2m from baseline to contracting for intact, exposed R, and exteriorized L muscles.

**Fig. 4.** Dynamic response of spinotrapezius (PO2m) across switches in inspired gas from left to right: 21% O2 (room air), 100, 21, and 10% O2 for a representative exteriorized muscle.
each capillary is elevated (17), thereby increasing RBC number along the fiber surface. However, it is now evident that, at least in some muscles, the majority of capillaries may be “recruited” at rest (4, 12, 15, 33, 42), which limits the opportunity for additional capillary recruitment during exercise. Thus the increased muscle O2-diffusing capacity from rest to exercise cannot rely greatly on the recruitment of additional capillaries or capillary units per se. Rather, it is likely determined by factors related to augmentation of RBC flow within previously RBC-perfused capillaries (2) or intracellular events (11). The present investigation demonstrates that surgical exteriorization does not elevate muscle blood flow nor alter microvascular Po2 at rest. These findings support the notion that the high percentage of capillaries supporting RBC flow reported in the spinotrapezius preparation (12, 14, 16, 33) is not an artifact secondary to elevated blood flow resulting from muscle manipulation or damage.

As mentioned earlier (see introduction), disruption of the distal blood supply lowers arterial and arteriolar blood pressure in the cremaster muscle (5). Indeed, for exteriorized muscles in general the feed artery pressures are substantially lower than those measured in intact muscles (5). Given that perfusion pressures are depressed by the exteriorization procedure, unless there was a concomitant vasodilatation, it would be expected that blood flow would be reduced. Interestingly, the report by DeLano and colleagues (5) demonstrates that proximal feed artery diameter is ~50% greater on average in the exteriorized cremaster muscle. Such a vasodilatory effect would act to counter any blood flow reduction consequent to decreased perfusion pressure. Given the above, it is possible that the unchanged blood flow found in the exteriorized spinotrapezius muscles in the present investigation represents the net effect of the surgical procedure on perfusion pressure (decrease) in combination with an arterial and arteriolar vasodilatation. It is also pertinent that the blood flow measurements made herein represent the average over the whole muscle and may therefore mask an altered regional distribution of that flow within the muscle. To perform regional blood flow measurements in a muscle as small as the rat spinotrapezius would have necessitated increasing the number of microspheres and risked compromising the preparation.

One potential effect of disrupting the distal blood supply would be the reduction of blood flow and likely microvascular Po2 under conditions that require a blood flow response close to peak. This effect would be expected to lower vascular conductance, blood flow, and microvascular Po2 in proportion to the relative capacity of the distal blood supply. That this was not observed herein reflects the modest level of metabolic stimulation evoked by DNP and the muscle stimulation paradigm utilized.

**Metabolic stimulation by DNP.** Muscle metabolic stimulation by DNP infusion elevates muscle VO2 approximately threefold (3), and DNP superfusion of the exteriorized hamster cremaster muscle increases arteriolar diameter and RBC velocity (39). There is evidence that this occurs via venular-arteriolar diffusion of vasoactive metabolites (38) rather than by a direct DNP-induced relaxation of arteriolar smooth muscle (3). In the present investigation, blood flow increased to 2.2-fold resting levels in both intact and exteriorized muscles, demonstrating that the net effect of DNP on the control of muscle blood flow was unaltered by surgical exteriorization.

It should be noted that mean blood flows for R and L spinotrapezius muscles were quite variable between groups 1 and 2 (see Figs. 1 and 2). The close similarity in blood flows to R and L muscles within each group under each condition suggests that there may have been subtle differences in anesthetic plane or other factors independent of surgical state that affected blood flows bilaterally.

**Electrically stimulated muscle contractions.** Oxygen delivery (QO2) to contracting myocytes is elevated by augmenting muscle blood flow and fractional O2 extraction. The precise regulators of the increased muscle blood flow are likely contingent on multiple factors such as the intensity, frequency, and duration of the contractions as well as the type of muscle (architecture, fiber type composition, oxidative capacity) and presiding experimental conditions. Given this, arterial driving pressure, muscle pump activity, endothelial cell shear stress, buildup of vasoactive metabolites, and reduced intramuscular Po2 are each thought to play a role in augmenting muscle blood flow during exercise. In addition, fractional O2 extraction increases as a hyperbolic function of muscle VO2 (32). During exercise, blood flow (and QO2) and O2 extraction are regulated tightly such that VO2 delivery increases in proportion to muscle VO2. The measurements of microvascular Po2 made in the present investigation assessed the VO2-to-QO2 relation at rest and during exercise. Thus, whereas neither VO2 nor QO2 themselves were measured, the finding of an unchanged microvascular Po2 between intact and exteriorized muscles at rest...
and the magnitude of the fall during contractions is indicative that, irrespective of the exact control mechanisms, the matching of VO₂ to QO₂ was not altered by surgical exteriorization.

**Hypoxic and hyperoxic switches.** Altered local PO₂ exerts a profound vasodilatory (hypoxia) or vasoconstrictory (hyperoxia) response (4, 23, 28). Because acute changes in inspired O₂ do not appear to alter pulmonary or tissue VO₂ appreciably at rest or during submaximal exercise (17), any subsequent alteration of microvascular PO₂ will depend on the effect of the inspired O₂ on arterial PO₂ (and O₂ content), MAP, and muscle vascular conductance, i.e., arteriolar smooth muscle tone (either modulated locally or neurally in response to central effects of hypo- or hyperoxia). Neither arterial PO₂ nor MAP was significantly different between intact and exteriorized preparations (same rats, order randomized). Hence the similarity of microvascular PO₂, with respect to the magnitude of the absolute change and dynamic profile of microvascular PO₂ across each switch, demonstrate that O₂-mediated arteriolar vasoaction was unaltered by surgical exteriorization.

In conclusion, the present investigation has demonstrated that the surgical exteriorization requisite for transmission intravital microscopy of the rat spinotrapezius muscle does not perturb significantly those key facets of integrated microcirculatory function measured in the present investigation. This is true at rest, in response to elevated metabolic rates (DNP, electrically induced muscle contractions), and systemic hypoxic or hyperoxic challenges.

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