Microvascular injury after ischemia and reperfusion in skeletal muscle of exercise-trained rats

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SEXTON, WILLIAM L., RONALD J. KORTHUIS, AND M. HAROLD LAUGHLIN. *Microvascular injury after ischemia and reperfusion in skeletal muscle of exercise-trained rats.* J. Appl. Physiol. 68(6): 2329–2336, 1990.—Ischemia and reperfusion in skeletal muscle is associated with increases in total vascular resistance (Rt) and the microvascular permeability to plasma proteins. To determine whether exercise training can attenuate ischemia and reperfusion-induced microvascular injury in skeletal muscle, intact (with skin) and skinned, maximally vasodilated (papaverine), isolated hindquarters of control (C) and exercise-trained (ET) rats were subjected to ischemia (intact 120 min; skinned 60 min) followed by 60 min of reperfusion. ET rats ran on a motorized treadmill at 32 m/min (8% grade), 2 h/day for 12 wk, whereas the C rats were cage confined. Before ischemia, ET hindquarters had higher isogravimetric flow, lower Rt, and similar solvent drag reflection coefficients (σ_f) compared with C. During reperfusion in intact hindquarters, flow was higher ($P < 0.05$) and Rt tended to be lower (15 ± 2 vs. 25 ± 5 mmHg·ml^{-1}·min·100 g; $P < 0.1$) in ET compared with C; however, in skinned hindquarters flow and Rt (14 ± 2 vs. 13 ± 2 mmHg·ml^{-1}·min·100 g) were not different between C and ET. During reperfusion, σ_f was reduced ($P < 0.05$) in both intact (C 0.68 ± 0.03; ET 0.68 ± 0.02) and skinned (C 0.66 ± 0.03; ET 0.68 ± 0.03) hindquarters, indicative of an increased microvascular permeability to plasma proteins. These results indicate that exercise training did not attenuate the microvascular injury (increased Rt and decreased σ_f) associated with ischemia and reperfusion in rat skeletal muscle.

isolated rat hindquarters; solvent drag reflection coefficient; microvascular permeability; vascular resistance; no-reflow phenomenon; muscle blood flow; microspheres

MUSCULAR ISCHEMIA is a long-standing clinical problem and can occur in conditions such as atherosclerosis, diabetes mellitus, circulatory shock, compartment syndrome, and traumatic injury. Reperfusion of ischemic muscle results in mitochondrial, sarcolemmal, and myofibrillar disruption (8, 23, 33), increased lipid oxidation (8), reductions in transmembrane potential (26), and vascular damage (17, 18, 22, 32, 33). A critical component of the pathology associated with ischemia-reperfusion injury in skeletal muscle is microvascular dysfunction (3, 17, 18). Microvascular injury associated with ischemia and reperfusion is characterized by an increased permeability to plasma proteins (3, 17, 18, 29), edema formation (29, 32, 33), and increased vascular resistance (17, 18, 29). Some regions of ischemic muscle fail to reperfuse upon reinstitution of flow, thus demonstrating the "no-reflow" phenomenon (33).

There is a growing body of evidence implicating reactive oxygen metabolites in the development of microvascular injury in skeletal muscle following ischemia and reperfusion (16–18). The activities of endogenous scavengers of reactive oxygen metabolites, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), appear to be correlated to the muscle fiber type composition (1, 9, 21) and the oxidative capacity of skeletal muscle (12, 21). Recently, it has been reported that, in addition to increasing the oxidative capacity of skeletal muscle, exercise training may also increase the activity of one or more of these endogenous scavengers in skeletal muscle (4, 9, 11, 13, 14, 21) and blood (15). Based on these observations, we hypothesized that exercise training may attenuate the microvascular injury observed in skeletal muscle during reperfusion following a period of prolonged ischemia.

The purpose of this study was to establish whether an intense regimen of endurance exercise training would attenuate the microvascular injury observed in skeletal muscle following ischemia and reperfusion. The effects of ischemia and reperfusion on capillary permeability to plasma proteins, total vascular resistance, and the regional distribution of blood flow among and within the hindlimb muscles of sedentary control and endurance-trained rats were assessed using the isolated perfused rat hindquarters model of ischemia and reperfusion injury as previously described (29). We have previously determined that 120 min of ischemia followed by 60 min of reperfusion produces microvascular injury that is consistently detectable in intact (with skin) rat hindquarters (29).

METHODS

Experimental design. Our initial design called for the comparison of the effects of ischemia and reperfusion on hemodynamic parameters, capillary filtration coefficient, the solvent drag reflection coefficient for plasma proteins, and the regional distribution of blood flow in isolated perfused hindquarters of sedentary control and exercise-trained rats as described below. After completion of an initial series of experiments, we observed that, during reperfusion, blood flow (measured using radiolabeled microspheres, see below) was redistributed so that

the cutaneous tissues received 30–40% of the total flow rather than the usual 5–10%. Since this relatively large proportion of total flow to the cutaneous bed could potentially "mask" the responses of the skeletal muscle microvasculature to ischemia and reperfusion (29), similar experiments were conducted using skinned hindquarters.

Experimental animals. Forty-seven male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), 200–250 g initial wt, were used in these experiments. The rats were housed two per cage (8 × 7 × 17 in.) under controlled temperature (22 ± 2°C) and light (12-h light-dark cycle) conditions. The rats were allowed free access to water and commercial rat chow.

Exercise-training program. All animals were initially subjected to light treadmill exercise to ensure that the animals to be used in the study were capable of running on the treadmill. After eliminating those animals that would not run, an investigator not directly involved in this study randomly assigned the remaining animals to either a sedentary control group (C) or an exercise-trained (ET) group. Animals in the ET group were subjected to a 12-wk 5 day/wk program of endurance exercise on a motorized rodent treadmill (modified Stanhope Scientific, Davis, CA). Treadmill elevation was 8% throughout the entire program. Exercise time and running speed were increased progressively during the first 6 wk to 120 min/day and 32 m/min, respectively, and maintained at these levels through the 12th wk of the program. This exercise-training program was modeled after that described by Holloszy (10), except no sprints were performed during the last weeks of the training program.

Hindquarters preparation. The rats were anesthetized using pentobarbital sodium (65 mg/kg ip), and a cannula (PE-240) was placed in the trachea to ensure a patent airway during surgery. A catheter (PE-50) was placed in the caudal artery for monitoring arterial pressure. The surgical preparation of the isolated perfused rat hindquarters was the same as described previously (19, 20, 28). The vena cava was cannulated with PE-240 tubing, and the venous effluent was directed to the perfusate reservoir. The aorta was cannulated with PE-160 tubing and perfused using a peristaltic pump (Gilson Minipuls 2). Total time for the cannulation procedure was usually <2 min, and interruption of flow to the hindquarters was usually <15 s. A tight ligature was placed around the lumbar musculature at the level of the catheters, and the hindquarters were separated from the body cephalad to the ligature.

Arterial perfusion pressure was monitored from the caudal artery catheter, and venous pressure was monitored from a side branch of the venous catheter using pressure transducers (Statham P23AC). The hindquarters preparation was placed on a grid suspended from a strain gauge transducer (Grass FT03C) to measure weight changes during the experiments. Arterial and venous pressures and hindquarters weight were recorded continuously on a Grass model 7D polygraph. The sensitivity of the weight-recording system was adjusted so that a 1-g weight produced a deflection of 2.5–3.0 cm on the recording paper. Rectal temperature was monitored using a telethermistor (Yellow Springs Instrument) and maintained at 37°C using an infrared heat lamp.

Skinned hindquarters preparation. In experiments using skinned rat hindquarters, the skin of deeply anesthetized rats was carefully removed from the hindquarters using thermal cautery before the surgical procedures described above. The cautery was applied to the dermal surface of the retracted skin to avoid contact with the underlying muscles. Superficial vessels supplying the skin were effectively cauterized during this procedure, and little or no hemorrhage was observed during the experiments. The hindquarters were coated with mineral oil and covered with plastic wrap to minimize desiccation during the experiment. As described previously (29), 60 min of ischemia followed by 60 min of reperfusion in skinned rat hindquarters result in microvascular injury of a similar magnitude to that observed in intact (with skin) hindquarters after 120 min of ischemia and 60 min of reperfusion.

Perfusate. The hindquarters were perfused with fresh human blood cells (American Red Cross Blood Donor Center, Columbia, MO) suspended in horse serum (Pel-Freeze). To prepare the blood cells, whole blood was centrifuged (10 min at 2,000 rpm) and the plasma supernatant was removed. The cells were resuspended in fresh saline and centrifuged, and the saline wash drawn off. This procedure was repeated three times before the cells were suspended in horse serum (protein concentration of 6 g/dl) with glucose (5.5 mM) and heparin (1,000 IU/160 ml). The cells were washed <24 h before each experiment and stored at 4°C. At the outset of each experiment, papaverine (average of 15 mg in 40–50 ml of perfusate) was titrated into the perfusate reservoir to achieve and maintain maximal vasodilation of hindquarters. Maximal vasodilation was presumed when the addition of 1 mg of papaverine caused no further reduction in perfusion pressure at the same flow rate. The venous effluent was directed back to the perfusate reservoir for recirculation and was bubbled with 95% O_2-5% CO_2, stirred continuously, and maintained at 37°C.

Citrate synthase activity. Citrate synthase activity was determined in the medial head and the deep red and the superficial white portions of the long head of the triceps brachii muscles. After dissection the muscles were immediately frozen in liquid N_2 and stored at −70°C. The muscle samples were homogenized on ice in 0.4 M KCl in 20% ethanol and centrifuged (23,000 *g*), and the supernatant was dialyzed against 2 mM phosphate buffer (pH = 7.4). Citrate synthase activity of the dialyzed supernatant was determined spectrophotometrically (412 nm) at 25°C as described by Srere (31).

Measurements. Isogravimetric capillary pressure (Pc,i) was determined by the isogravimetric method of Pappenheimer and Soto-Rivera (25). Flow to the hindquarters and venous pressure were adjusted to achieve the isogravimetric state (i.e., no net weight change). To determine Pc,i, flow was reduced a small increment, which resulted in a fall in perfusion pressure and hindquarters weight. Venous pressure was then increased to maintain the isogravimetric state. This procedure was repeated four

or five times. Pc,i was calculated from the linear regression relationship between isogravimetric flow and venous pressure and is equal to the zero intercept of the venous pressure axis (25).

Total vascular resistance (Rt), precapillary vascular resistance (Ra), and postcapillary vascular resistance (Rv) were calculated according to the relationships; Rt = (Pa,i − Pv,i)/$\dot{Q}$i, Ra = (Pa,i − Pc,i)/$\dot{Q}$i, and Rv = (Pc,i − Pv,i)/$\dot{Q}$i, where Pa,i and Pv,i are the isogravimetric arterial and venous pressures, respectively, and $\dot{Q}$i is the isogravimetric flow. The pre-to-postcapillary resistance ratios were also calculated.

The capillary filtration coefficient was determined as described by Pappenheimer and Soto-Rivera (25). From the isogravimetric state, venous pressure was increased ~10 mmHg. The temporal pattern of weight gain in the hindquarters consisted of an initial fast component, representing vascular pooling, followed by a slow component, representing fluid filtration from the capillaries into the interstitium (25). The rate of fluid filtration in milliliters per minute per 100 g was calculated from the rate of slow weight gain between 2 and 4 min. The capillary filtration coefficient was calculated by dividing the rate of fluid filtration by 85% of the increment in venous pressure, which approximates the increase in mean capillary pressure (20, 28, 29).

The solvent drag reflection coefficient for total plasma proteins (σ_f) was estimated using the integral mass balance modification (34) of the filtered volumes technique (24). With this method, the erythrocytes serve as the nondiffusible intravascular marker and the plasma proteins serve as the diffusible marker (24, 34). Before the determination of σ_f, the reservoir volume was reduced to 15–20 ml. The perfusate hematocrit and total protein concentration were determined in quadruplicate before and after a period of fluid filtration induced by elevation of the venous pressure by ~20 mmHg. Hematocrit was determined by the microhematocrit method, and the protein concentration of the plasma supernatant was determined using a clinical refractometer (American Optical). Filtration was allowed to continue for 15–20 min to ensure that the changes in hematocrit and total plasma protein concentration were greater than the error incurred in their measurement. σ_f was calculated using the relationship (34)

$$\sigma_f = 1 - \left\{\left[\frac{C_i}{(C_i + C_f)/2}\right] 1 - \left[\frac{(1 - H_i)\ (1 - C_i/C_f)}{(1 - H_i/H_f)}\right]\right\}$$

where H_i and H_f are the initial and final hematocrits and C_i and C_f are the initial and final plasma protein concentrations, respectively. Perfusate removed before determination of σ_f was returned to the reservoir, and ~15 min was allowed for restabilization of the preparation.

Determination of regional distribution of flow. The regional distribution of flow within hindquarters was determined during reperfusion using radiolabeled (^{141}Ce or ^{103}Ru) microspheres (16.5 ± 0.1 μm, NEN-Trac Microspheres, NEN Research Products, Boston, MA) as described previously (19, 20, 29). The microspheres were suspended in saline with 10% dextran and 0.01% Tween 80. Before infusion, the microspheres were sonicated and well mixed using an electromagnetic stirrer and a stir bar contained within the microspheres suspension vial. The microsphere suspensions (0.1 ml, ~200,000 microspheres) were slowly infused over 15–20 s into a mixing chamber interposed in the arterial line immediately proximal to the hindquarters. Reference withdrawal samples from the caudal artery were begun 15–20 s before the infusion of microspheres and continued for 2 min after the infusion. Immediately after each experiment, the hindquarters were dissected and tissue samples (soleus, vastus intermedius, and red, mixed, and white portions of the gastrocnemius and the vastus lateralis, skin, and feet) were collected and weighed for analysis of regional flows. The radioactivity of the tissues was determined using a gamma counter (Tracor Analytic 2250). Tissue flows (ml·min^{-1}·100 g^{-1}) were calculated from the ratio of the counts per minute in the tissue samples and the reference withdrawal sample multiplied by the reference withdrawal flow rate and normalized per 100 g of tissue.

Experimental protocol. After the institution of flow to the hindquarters, flow and venous pressure were adjusted to establish an isogravimetric state, and papaverine was titrated into the reservoir to achieve maximal vasodilation. The capillary filtration coefficient was determined twice 30–40 min after the initiation of perfusion, after which Pc,i and σ_f were determined. Ischemia was produced by turning off the perfusion pump and simultaneously clamping both the arterial and venous catheters just proximal to the aorta and vena cava. Perfusate within the arterial and venous lines was flushed into the reservoir with small volumes of saline to prevent settling and clumping of the blood cells within the tubing during the ischemic period. Normothermic (37°C) ischemia was maintained for 120 min for intact hindquarters and 60 min for skinned hindquarters. Before reperfusion, the arterial lines were primed with perfusate from the reservoir. Reperfusion was initiated with low flows (<1 ml/min) to avoid the possibility of vascular damage associated with the sudden introduction of high flows. Flow was increased during the initial 10–15 min of reperfusion to attain an isogravimetric state. Papaverine (2–3 mg) was again titrated into the reservoir to ensure maximal vasodilation. The capillary filtration coefficient was determined 30–40 min after the initiation of reperfusion, and total vascular resistance and σ_f were measured after 60 min. Pc,i was not determined during reperfusion due to the low isogravimetric flows. Ten to 15 min after the determination of σ_f, flow was increased to preischemia isogravimetric levels and microspheres were infused. The hindquarters were then dissected, and tissue samples were taken for the assessment of the regional distribution of flow.

Statistical analysis. The data are expressed as means ± SE. Hemodynamic, capillary filtration coefficients, and σ_f data were compared using a Student's *t* test. Tissue blood flow data were compared using a one-way analysis of variance and Duncan's new multiple range test where appropriate. $P \leq 0.05$ was considered to be significant.

RESULTS

Citrate synthase activity. As shown in Table 1, citrate synthase activities of the medial head and both the deep

TABLE 1. *Citrate synthase activities of triceps brachii muscles from control and exercise-trained rats*

	n	TM	TLR	TLW
Control	7	48.4±0.3	24.7±0.2	22.8±0.2
Trained	10	65.2±0.5*	62.4±0.8*	29.1±0.2*

Values of citrate synthase activity are means ± SE expressed in U/mg protein. TM, medial head of triceps brachii muscle; TLR and TLW, deep red and superficial white portions of long head of triceps brachii muscle, respectively. * Significant difference from control, $P \leq 0.05$.

TABLE 2. *Preischemia hemodynamic data for intact and skinned hindquarters of control and exercise-trained rats in the maximally vasodilated isogravimetric state*

	Intact		Skinned	
	Control	Trained	Control	Trained
n	7	10	16	14
Flow, ml·min^{-1}·100 g^{-1}	9.0±0.4	11.9±0.4*	11.9±0.4	13.4±0.3*
Arterial pressure, mmHg	37.6±1.5	37.1±0.9	39.2±1.0	39.2±0.9
Venous pressure, mmHg	2.1±0.2	2.2±0.3	1.5±0.4	1.0±0.3
Perfusion pressure, mmHg	35.5±1.4	34.9±1.0	37.7±0.8	38.2±0.8
Capillary pressure, mmHg	13.7±0.9	12.2±0.5	13.4±0.4	13.4±0.6
Vascular resistance, mmHg·ml^{-1}·min·100 g				
Total	4.0±0.1	3.0±0.2*	3.2±0.1	2.8±0.1*
Precapillary	2.7±0.1	2.1±0.1*	2.2±0.1	1.9±0.1*
Postcapillary	1.3±0.1	0.9±0.1*	1.0±0.1	0.9±0.1
Pre-to-postcapillary resistance ratio	2.1±0.3	2.6±0.3	2.3±0.2	2.2±0.2

Values are means ± SE. * Significant difference from control, $P \leq 0.05$.

and superficial portions of the long head of the triceps brachii muscles were increased in ET rats compared with C.

Preischemia hemodynamic data. Preischemia isogravimetric data for maximally vasodilated intact and skinned hindquarters of C and ET rats are presented in Table 2. Arterial, venous, and perfusion (arterial minus venous) pressures were not different between C and ET groups. Capillary pressures were also not different between groups. However, isogravimetric flows were higher in ET hindquarters. In addition, ET hindquarters showed reductions in both total and precapillary vascular resistances compared with C. The postcapillary vascular resistance of the ET group was lower in the intact hindquarters but was not different from C in the skinned preparations. Pre-to-postcapillary resistance ratios were not different between C and ET groups.

Postischemia-reperfusion hemodynamic data. The effects of ischemia and reperfusion on isogravimetric perfusion pressures, flow, and total vascular resistances in maximally vasodilated intact C ($n = 7$) and ET ($n = 10$) rat hindquarters are presented in Fig. 1. During reperfusion, isogravimetric perfusion pressures tended to be elevated over preischemia levels ($P < 0.2$) in both C and ET hindquarters. In contrast, isogravimetric flows were lower compared with preischemia levels (Fig. 1), with the ET hindquarters having higher flows than C, 2.9 ± 0.2 vs. 1.8 ± 0.3 ml·min^{-1}·100 g^{-1}, respectively. Total vascular resistances were increased in both C and ET during reperfusion (Fig. 1) and tended to be higher in C compared with ET hindquarters (24.3 ± 4.6 vs. 14.9 ± 2.2 mmHg·ml^{-1}·min·100 g, respectively), but this difference was not significant ($P < 0.10$).

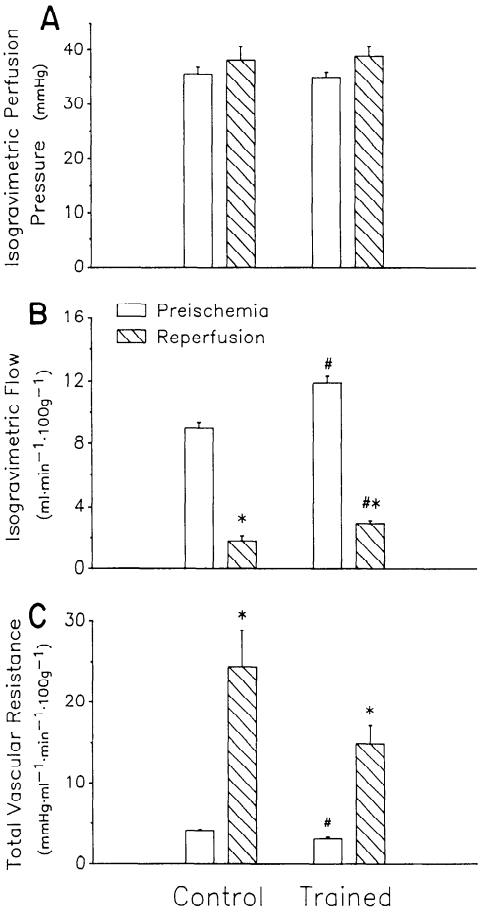

FIG. 1. Perfusion pressure (*A*), flow (*B*), and total vascular resistance (*C*) data determined under isogravimetric conditions before ischemia (preischemia) and during reperfusion in intact hindquarters of control ($n = 7$) and exercise-trained ($n = 10$) rats. Values are means ± SE. # Significant difference between control and trained groups, $P \leq 0.05$. * Significant difference from preischemia, $P \leq 0.05$.

The effects of ischemia and reperfusion on hemodynamics in the skinned hindquarters were qualtitatively similar to those seen in the intact hindquarters (Table 3). Isogravimetric perfusion pressures during reperfusion were elevated over preischemia levels but were not different between the skinned C and ET groups. Isogravimetric flows in skinned hindquarters were lower after ischemia and reperfusion compared with preischemia. However, in contrast to the intact hindquarters, postischemic flows were similar in the skinned C and ET hindquarters. Total vascular resistance was increased in skinned C and ET hindquarters during reperfusion by 322 ± 52 and 360 ± 71%, respectively. These increases were comparable to those seen in the intact hindquarters (508 ± 100 and 385 ± 50% for C and ET, respectively).

Solvent drag reflection coefficient data. Figure 2 depicts the data for σ_f determined before ischemia and during reperfusion after ischemia in intact hindquarters of C ($n = 7$) and ET ($n = 10$) rats. Preischemia values for σ_f

TABLE 3. *Hemodynamic data obtained for skinned hindquarters of control and trained rats before ischemia and during reperfusion after ischemia*

		Preischemia	Reperfusion
Flow, ml·min^{-1}·100 g^{-1}	C	11.9±0.4	3.8±0.5†
	T	13.4±0.3*	3.8±0.4†
Perfusion pressure, mmHg	C	37.7±0.8	40.3±0.8†
	T	38.2±0.8	41.9±1.3†
Total vascular resistance, mmHg·ml^{-1}·min·100 g	C	3.2±0.1	13.5±1.7†
	T	2.8±0.1*	12.9±2.0†
Solvent drag reflection coefficient	C	0.79±0.01	0.66±0.03†
	T	0.82±0.02	0.68±0.03†
Capillary filtration coefficient, ml·min^{-1}·mmHg^{-1}·100 g^{-1}	C	0.0166±0.0008	0.0132±0.0010†
	T	0.0197±0.0009*	0.0137±0.0013†

Values are means ± SE. C, control (n = 15); T, trained (n = 11). * Significant difference from C, $P \leq 0.05$. † Significant difference from preischemia values, $P \leq 0.05$.

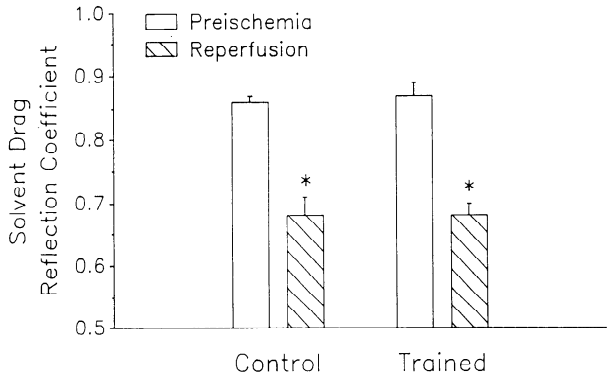

FIG. 2. Solvent drag reflection coefficient data determined before ischemia (preischemia) and during reperfusion in hindquarters of control (n = 7) and exercise-trained (n = 10) rats. Values are means ± SE. * Significant difference from preischemia, $P \leq 0.05$.

were not different between C (0.86 ± 0.01) and ET (0.87 ± 0.02) hindquarters. After ischemia and reperfusion, σ_f values were reduced to a similar extent in both C (0.68 ± 0.03) and ET (0.68 ± 0.02) hindquarters, indicative of an increase in the microvascular permeability to plasma proteins. As shown in Table 3, qualitatively similar responses were noted in skinned preparations.

Capillary filtration coefficient data. Preischemia capillary filtration coefficients for intact hindquarters of ET rats were greater compared with C, 0.0218 ± 0.0017 vs. 0.0169 ± 0.0015 ml·min^{-1}·mmHg^{-1}·100 g^{-1}, respectively. After ischemia and reperfusion, filtration coefficients for C hindquarters (0.0147 ± 0.0020) were similar to preischemia values, whereas filtration coefficients for ET hindquarters (0.0154 ± 0.0013) were lower relative to preischemia. In the skinned hindquarters, filtration coefficients for both C and ET groups were lower after ischemia and reperfusion (Table 3).

Reperfusion distribution of flow. Hemodynamic values and blood flows to selected hindlimb muscles, skin, and feet measured during reperfusion in both intact and skinned hindquarters are presented in Table 4. Immediately before the infusion of microspheres during reperfusion, total hindquarters flow was increased to the preischemia isogravimetric level to obtain adequate flow to ensure delivery of the microspheres to the perfused tissues. At these flows, the perfusion pressures and total vascular resistances were nearly doubled compared with preischemia values (Table 4). No differences were found in postischemic skeletal muscle blood flows between C and ET (Table 4). In C and ET hindquarters of both the intact and skinned groups, flows were highest in those muscles composed of a large percentage of slow-twitch oxidative (SO) and fast-twitch oxidative-glycolytic (FOG) muscle fibers, such as the soleus, vastus intermedius, and the deep red portion of the gastrocnemius (2) muscles. Conversely, the lowest flows were observed in muscles composed primarily of fast-twitch glycolytic (FG) muscle fibers, such as the superficial white portions of the gastrocnemius and the vastus lateralis muscles (2).

Flows to the skin and feet of intact hindquarters were not different between C and ET groups during reperfusion (Table 4), accounting for 17 ± 4 and 19 ± 8% of the total flow in C and 22 ± 8 and 16 ± 7% of the total flow in ET hindquarters, respectively. In skinned hindquarters, flow to the feet accounted for 11 ± 3 and 9 ± 2% of the total flow in C and ET rats, respectively. These data indicate that ischemia and reperfusion had little effect on blood flow to the cutaneous tissues, since the relative flows are similar to those reported in the literature (5, 29).

DISCUSSION

Recent evidence indicates that the microvascular injury observed during the reperfusion of ischemic skeletal muscle is mediated, at least in part, by reactive oxygen metabolites generated during the period of reperfusion (16–18). Moreover, pretreatment of skeletal muscle with substances that inhibit oxidant production or that effectively scavenge oxidants at the time of production attenuates the microvascular injury observed following ischemia and reperfusion (18). Skeletal muscle contains a number of endogenous antioxidant enzymes, such as SOD, CAT, and GPX, that effectively scavenge reactive oxygen metabolites and may help protect the muscle from oxidant injury (1, 11, 12, 30). The activities of endogenous SOD, CAT, and GPX differ among skeletal muscles of differing muscle fiber type compositions (1, 9, 21) and the oxidative capacities (11, 21). Exercise training, which increases the oxidative capacity of skeletal muscle, has also been shown to alter the activities of these endogenous antioxidants (1, 4, 9, 11, 13, 14, 21). As shown in the accompanying paper (21), the exercise-training program used in this study produced increases in the GPX activity of the leg extensor muscles of these rats. An increase in skeletal muscle GPX activity could enhance the resistance to injury following ischemia and reperfusion. Thus we hypothesized that exercise training would attenuate the microvascular injury observed in skeletal muscle following ischemia and reperfusion.

Effects of exercise training. Exercise training resulted in increased citrate synthase activities in skeletal muscles representative of all muscle fiber types (Table 1), increased isogravimetric flows, lower minimal vascular resistances, and increased capillary filtration coefficients. Thus the skeletal muscle of the exercise-trained rats used in this study showed evidence of both biochem-

TABLE 4. *Hemodynamic data obtained immediately before infusion of microspheres during reperfusion and regional blood flows for intact and skinned hindquarters of control and trained rats*

	Intact		Skinned	
	Control	Trained	Control	Trained
Total hindquarters hemodynamics				
n	6	9	11	9
Flow, ml·min^{-1}·100 g^{-1}	8.9±0.5	11.8±0.5*	12.1±0.4	13.2±0.3*
Perfusion pressure, mmHg	67±5	67±4	68±5	72±4
Vascular resistance, mmHg·ml^{-1}·min·100 g	7.4±0.4	5.6±0.5*	5.7±0.5	5.4±0.3
Regional blood flows, ml·min^{-1}·100 g^{-1}				
Soleus	44±25	32±14	78±19	43±6
Gastrocnemius, red	25±7	28±11	47±13	36±8
Gastrocnemius, mixed	15±6	18±9	17±4	15±4
Gastrocnemius, white	9±3	13±8	9±2	6±1
Vastus intermedius	18±4	24±9	36±9	21±3
Vastus lateralis, red	5±2	12±5	11±3	12±3
Vastus lateralis, mixed	12±5	14±5	16±4	17±4
Vastus lateralis, white	8±3	7±2	7±2	6±1
Skin	20±11	14±5		
Feet	54±21	48±19	31±8	25±6

Values are means ± SE. * Significant difference from control, $P \leq 0.05$.

ical and vascular adaptations similar to previous reports (10, 19, 20, 28).

Effects of ischemia and reperfusion. Microvascular injury associated with the reperfusion of ischemic skeletal muscle is characterized by increases in both the microvascular permeability to plasma proteins (decreased σ_f) and total vascular resistance (3, 16–18, 29). As shown in Figs. 1 and 2 and Table 3, ischemia and reperfusion produced the expected effects on σ_f and total vascular resistance in hindquarters of both control and exercise trained rats. The reductions in σ_f observed after ischemia and reperfusion were not different between control and trained hindquarters. Although the isogravimetric blood flow data during reperfusion suggest a degree of protection in the intact hindquarters of trained rats, since flows were 61% greater and total vascular resistances were 39% less than in the controls, the relative reduction in flow noted following ischemia and reperfusion was similar for the control (79 ± 4%) and trained (76 ± 2%) groups. Thus it appears that the relative magnitude of the no-reflow phenomenon noted in postischemic rat skeletal muscle was not altered by exercise training.

As shown in Table 3, σ_f was reduced a similar amount following ischemia and reperfusion in skinned hindquarters of control and exercise trained rats. In addition, there was no evidence of protection following ischemia and reperfusion in the skinned hindquarters of trained rats, since isogravimetric flows and total vascular resistances were similar in control and trained hindquarters (Table 3). These results suggest that exercise training does not attenuate the microvascular injury associated with 60 (skinned) or 120 (intact) min of ischemia and 60 min of reperfusion in rat skeletal muscle.

The capillary filtration coefficient represents the product of the surface area available for fluid exchange and the hydraulic conductivity of the exchange vessels. The increases in total and regional vascular resistances after ischemia and reperfusion indicate that there was a reduction in the perfused surface area which contributed to the reductions in filtration coefficient. However, it is also possible that decreases in σ_f may be associated with increases in the hydraulic conductivity of the exchange vessels. The net capillary filtration coefficient following ischemia and reperfusion could represent both the effects of a reduced surface area available for exchange and an increased permeability of the exchange vessels to protein and, perhaps, water (17, 29). Thus caution is warranted in the use of capillary filtration coefficient data as an indicator of microvascular dysfunction following ischemia and reperfusion.

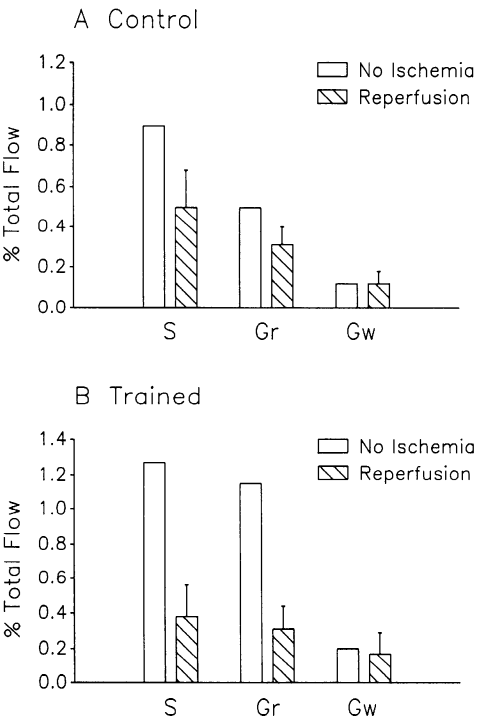

FIG. 3. Distribution of hindquarters flow to skeletal muscles of different muscle fiber type compositions (S, soleus; Gr, red gastrocnemius; Gw, white gastrocnemius) from intact hindquarters of control (*A*; n = 6) and exercise-trained (*B*; n = 9) rats. Nonischemia (no ischemia) values are averages calculated from the data of Laughlin and Ripperger (20). Reperfusion values for percentage of total flow after 120 min of ischemia and 60 min of reperfusion (means ± SE) were calculated from data presented in Table 4.

The pattern of the regional distribution of flow within and among hindlimb skeletal muscles during reperfusion was similar to that previously described in nonischemic maximally vasodilated rat hindquarters in that the blood flows tended to be directly correlated with the oxidative capacity of the muscles (19, 20). Postischemic blood flows were not different between control and trained rats in either the intact or skinned preparations for any of the muscles sampled (Table 4). Postischemic blood flows to the soleus and red, mixed, and white portions of the gastrocnemius of the control hindquarters were >50% lower than would be predicted from the data of Laughlin and Ripperger (20) for hindquarters of similar rats. Laughlin and Ripperger (20) also reported that an endurance-training program similar to the one used in the present study (31 m/min at 5° incline, 60 min/day, for 13–17 wk) resulted in increased flow to the soleus and red, mixed, and white portions of the gastrocnemius muscles averaging 130% over control flows. It is therefore likely that before ischemia both the soleus and gastrocnemius muscles of the trained hindquarters in this study received higher flows than the controls. Comparison of the postischemic muscle flows in control and trained hindquarters in this study with expected preischemia flows (20) suggests that the relative increases in vascular resistance observed after ischemia and reperfusion were greater in the trained muscles than in the controls.

In both the intact control and trained hindquarters, the percentages of total flow received by the soleus (89% SO fibers) and red gastrocnemius (30% SO and 56% FOG fibers) muscles were lower compared with the data of Laughlin and Ripperger (20) (Fig. 3). In contrast, the percentage of total flow received by the white gastrocnemius (91% FG fibers) was similar. These comparisons suggest that ischemia and reperfusion probably resulted in relatively greater increases in the vascular resistance of highly oxidative SO and FOG muscles than in FG muscles.

Although exercise training may produce increases in the activities of endogenous antioxidants (1, 9, 11, 13, 14, 21) in skeletal muscle, these changes did not appear to protect the hindquarters microvasculature from injury during ischemia and reperfusion. It is possible that the microvascular damage observed here was so great that the effects of training were not adequate to protect the hindquarters from injury. However, these experiments were based on preliminary experiments designed to determine the minimal duration of ischemia necessary to produce microvascular damage in rat hindquarters that was consistently detectable (29). We therefore believe that if exercise training were to provide protection to the microvasculature during ischemia and reperfusion, it would have been apparent here. In addition, the site of oxidant generation in postischemic skeletal muscle is not known. If reactive oxygen metabolites are produced primarily within the lumen of exchange vessels, the endothelium, or the interstitium, then increases in myocyte antioxidant activities may not protect the microvasculature. Also, the myoglobin content in SO and FOG muscles, but not FG muscles, is increased after exercise training in rats (7). In the presence of hydrogen peroxide, myoglobin can catalyze the formation of the hydroxyl radical (27) and/or a reactive oxidant possessing a similar reactivity (6). Increases in the myoglobin content of a muscle after training may contribute to oxidant injury observed following ischemia and reperfusion. Furthermore, although there is good evidence indicating that reactive oxygen metabolites are involved in the production of microvascular injury in skeletal muscle (16–18), other factors, such as neutrophilic proteases (30), may also be involved.

In summary, exercise training did not alter the susceptibility of skeletal muscle to microvascular injury associated with ischemia and reperfusion in isolated perfused rat hindquarters. Although it appears that exercise training does not make skeletal muscle more resistant to postischemic microvascular injury, it is important to emphasize that we have only investigated acute microvascular damage following ischemia and reperfusion. It remains possible that the long-term recovery of the skeletal muscle tissue is enhanced by exercise training.

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REFERENCES

1. ALESSIO, H. M., AND A. H. GOLDFARB. Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. *J. Appl. Physiol.* 64: 1333–1336, 1988.
2. ARMSTRONG, R. B., AND R. O. PHELPS. Muscle fiber type composition of the rat hindlimb. *Am. J. Anat.* 171: 259–272, 1984.
3. DIANA, J. N., AND M. H. LAUGHLIN. Effect of ischemia on capillary pressure and equivalent pore radius in capillaries of the isolated dog hindlimb. *Circ. Res.* 35: 77–101, 1974.
4. GIRTEN, B., C. OLOFF, P. PLATO, E. EVELAND, A. J. MEROLA, AND L. KAZARIAN. Skeletal muscle antioxidant enzyme levels in rats after simulated weightlessness, exercise and dobutamine. *Physiologist* 32, *Suppl.*: S59–S60, 1988.
5. GORSKI, J., D. A. HOOD, AND R. L. TERJUNG. Blood flow distribution in tissues of perfused rat hindlimb preparations. *Am. J. Physiol.* 250 (*Endocrinol. Metab.* 13): E441–E448, 1986.
6. GRISHAM, M. B. Myoglobin-catalyzed hydrogen peroxide dependent arachidonic acid peroxidation. *J. Free Radicals Biol. Med.* 1: 227–232, 1985.
7. HARMS, S. J., AND R. C. HICKSON. Skeletal muscle mitochondria and myoglobin, endurance, and intensity of training. *J. Appl. Physiol.* 54: 798–802, 1983.
8. HARRIS, K., P. M. WALKER, D. A. G. MICKLE, R. HARDING, R. GATELY, G. J. WILSON, B. KUZON, N. MCKEE, AND A. D. ROMASCHIN. Metabolic response of skeletal muscle to ischemia. *Am. J. Physiol.* 250 (*Heart Circ. Physiol.* 19): H213–H220, 1986.
9. HIGUCHI, M., L.-J. CARTIER, M. CHEN, AND J. O. HOLLOSZY. Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise. *J. Gerontol.* 40: 281–286, 1985.
10. HOLLOSZY, J. O. Adaptations of muscular tissue to training. *Prog. Cardiovasc. Dis.* 18: 445–458, 1976.
11. JENKINS, R. R. The role of superoxide dismutase and catalase in

muscle fatigue. In: *Biochemistry of Exercise*, edited by H. G. Knuttgen, J. A. Vogel, and J. Poortmans. Champaign, IL: Human Kinetics, 1983, vol. 13, p. 467–471.

12. JENKINS, R. R., R. FRIEDLAND, AND H. HOWALD. The relationship of oxygen uptake to superoxide dismutase and catalase activity in human skeletal muscle. *Int. J. Sports Med.* 5: 11–14, 1984.
13. JI, L. L., F. W. STRATMAN, AND H. A. LARDY. Enzymatic down regulation with exercise in rat skeletal muscle. *Arch. Biochem. Biophys.* 263: 137–149, 1988.
14. JI, L. L., F. W. STRATMAN, AND H. A. LARDY. Antioxidant enzyme systems in rat liver and skeletal muscle. *Arch. Biochem. Biophys.* 263: 150–160, 1988.
15. KANTER, M. M., R. L. HAMLIN, D. V. UNDERFERTH, H. W. DAVIS, AND A. J. MEROLA. Effect of exercise training on antioxidant enzymes and cardiotoxicity of doxorubicin. *J. Appl. Physiol.* 59: 1298–1303, 1985.
16. KORTHUIS, R. J., AND D. N. GRANGER. Ischemia-reperfusion injury: role of oxygen-derived free radicals. In: *Physiology of Oxygen Radicals*, edited by A. E. Taylor, S. Matalan, and P. A. Ward. Bethesda, MD: Am. Physiol. Soc., 1986, p. 217–249. (Clin. Physiol. Ser.)
17. KORTHUIS, R. J., D. N. GRANGER, M. I. TOWNSLEY, AND A. E. TAYLOR. The role of oxygen-derived free radicals in ischemia-induced increases in canine skeletal muscle microvascular permeability. *Circ. Res.* 57: 599–609, 1985.
18. KORTHUIS, R. J., J. K. SMITH, AND D. L. CARDEN. Hypoxic reperfusion attenuates postischemic microvascular injury. *Am. J. Physiol.* 256 (*Heart Circ. Physiol.* 25): H315–H319, 1989.
19. LAUGHLIN, M. H., R. J. KORTHUIS, W. L. SEXTON, AND R. B. ARMSTRONG. Regional muscle blood flow capacity and exercise hyperemia in high-intensity trained rats. *J. Appl. Physiol.* 64: 2420–2427, 1988.
20. LAUGHLIN, M. H., AND J. RIPPERGER. Vascular transport capacity of hindlimb muscles of exercise trained rats. *J. Appl. Physiol.* 62: 438–443, 1987.
21. LAUGHLIN, M. H., T. SIMPSON, O. R. BROWN, W. L. SEXTON, J. K. SMITH, AND R. J. KORTHUIS. Skeletal muscle oxidative capacity, antioxidant enzyme, and exercise training. *J. Appl. Physiol.* 68: 2337–2343, 1990.
22. MAKITIE, J. Microvasculature of rat striated muscle after temporary ischemia. *Acta Neuropathol.* 37: 247–253, 1977.
23. MAKITIE, J., AND H. TERAVAINEN. Ultrastructure of striated muscle of the rat after temporary ischemia. *Acta Neuropathol.* 37: 237–245.
24. MARON, M., AND C. F. PILATI. Calculation of the reflection coefficient from measurements of endogenous vascular indicators. *J. Appl. Physiol.* 64: 1746–1748, 1988.
25. PAPPENHEIMER, J. R., AND A. SOTO-RIVERA. Effective osmotic pressure of plasma proteins and other quantities associated with capillary circulation in the hindlimb of cats and dogs. *Am. J. Physiol.* 152: 471–491, 1948.
26. PERRY, M. O., AND G. FANTINI. Ischemia: profile of an enemy. *J. Vasc. Surg.* 6: 231–234, 1987.
27. PUPPO, A., AND B. HALLIWELL. Formation of hydroxyl radicals in biological systems. Does myoglobin stimulate hydroxyl radical formation from hydrogen peroxide? *Free Rad. Res. Commun.* 4: 415–422, 1988.
28. SEXTON, W. L., R. J. KORTHUIS, AND M. H. LAUGHLIN. High-intensity exercise training increases vascular transport capacity of rat hindquarters. *Am. J. Physiol.* 254 (*Heart Circ. Physiol.* 23): H274–H278, 1988.
29. SEXTON, W. L., R. J. KORTHUIS, AND M. H. LAUGHLIN. Ischemia-reperfusion injury in isolated rat hindquarters. *J. Appl. Physiol.* 68: 387–392, 1990.
30. SMITH, J. K., M. B. GRISHAM, D. N. GRANGER, AND R. J. KORTHUIS. Free radical defense mechanisms and neutrophil infiltration in postischemic skeletal muscle. *Am. J. Physiol.* 256 (*Heart Circ. Physiol.* 25): H789–H793, 1989.
31. SRERE, P. A. Citrate synthase. *Methods Enzymol.* 13: 3–5, 1969.
32. STROCK, P. E., AND G. MAJNO. Vascular responses to tourniquet ischemia. *Surg. Gynecol. Obstet.* 129: 309–318, 1969.
33. STROCK, P. E., AND G. MAJNO. Microvascular changes in acutely ischemic rat muscle. *Surg. Gynecol. Obstet.* 129: 1213–1224, 1969.
34. WOLF, M. B., P. D. WATSON, AND D. R. G. SCOTT. Integral-mass balance method for determination of the solvent drag reflection coefficient. *Am. J. Physiol.* 253 (*Heart Circ. Physiol.* 22): H194–H204, 1987.