Exercise Training Improves Insulin-Mediated Capillary Recruitment in Association With Glucose Uptake in Rat Hindlimb

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Exercise training is considered to be beneficial in the treatment and prevention of insulin insensitivity, and much of the effect occurs in muscle. We have recently shown that capillary recruitment by insulin in vivo is associated with and may facilitate insulin action to increase muscle glucose uptake. In the present study, we examined the effect of 14 days of voluntary exercise training on euglycemic-hyperinsulinemic clamped (10 mU · min^{-1} · kg^{-1} for 2 h), anesthetized rats. Whole-body glucose infusion rate (GIR), hindleg glucose uptake, femoral blood flow (FBF), vascular resistance, and capillary recruitment, as measured by metabolism of infused 1-methylxanthine (1-MX), were assessed. In sedentary animals, insulin caused a significant ($P <$ 0.05) increase in FBF (1.6-fold) and capillary recruitment (1.7-fold) but a significant decrease in vascular resistance. In addition, hindleg glucose uptake was increased (4.3-fold). Exercise training increased insulin-mediated GIR (24%), hindleg glucose uptake (93%), and capillary recruitment (62%) relative to sedentary animals. Neither capillary density nor total xanthine-oxidase activity in skeletal muscle were increased as a result of the training regimen used. We concluded that exercise training improves insulin-mediated increases in capillary recruitment in combination with augmented muscle glucose uptake. Increased insulin-mediated glucose uptake may in part result from the improved hemodynamic control attributable to exercise training. *Diabetes* **50:2659–2665, 2001**

It has been known for a number of years that physical training induces improvements of glucose tolerance in diabetic patients and a substantial lowering of serum cholesterol and triglyceride levels as well as a rise in HDL cholesterol and a decrease in body weight (1). Sedentary individuals generally have a low exercise capacity and rapidly fatigue (2). Physical training can reverse this situation and increase exercise capacity and endurance. Adaptations responsible for this include changes to the cardiovascular system and skeletal muscle,

which is largely responsible for the increases in exercise capacity and endurance that occur with training. The heart adapts with an increase in maximum stroke volume and cardiac output (3). As a consequence, the maximum capacity to provide the working muscles with blood and oxygen is increased. Skeletal muscles adapt to training with an increase in capillary supply (4), and a variety of enzymes and proteins are induced that permit increased capacity for aerobic metabolism. These include the enzymes of the citrate cycle (5) and mitochondrial respiratory chain (6); the enzymes in the activation, transport, and β-oxidation of long chain fatty acids (7); the enzymes involved in ketone oxidation (8); the enzymes of the malate aspartate shuttle (9); the mitochondrial coupling factor (10); and the myoglobin concentration (11). There is an increase in both the size and number of mitochondria in trained skeletal muscle (12). There is also evidence that exercise training increases GLUT4 mRNA and GLUT4 protein levels in animals (13,14) and humans (15). As a consequence of the increases in maximum cardiac output and in the capacity of skeletal muscle for aerobic metabolism induced by exercise training, exercise capacity and maximum oxygen uptake capacity are increased. Furthermore, exercise-trained individuals can undergo strenuous submaximal exercise without disturbance of homeostasis, as reflected in large lactate accumulation and glycogen depletion.

In terms of glucose homeostasis, exercise training induces an increase in GLUT4 expression in muscle, and this is argued to contribute to an increase in the responsiveness of muscle glucose uptake to insulin (16), along with other adaptations of glucose metabolism (17). A few studies have highlighted the hemodynamic adaptations resulting from exercise training that could alter the delivery of glucose and insulin to muscle (18,19). In one of these studies (19), it was noted that forearm blood flow in athletes was 64% greater than in sedentary subjects and was closely correlated with whole-body glucose disposal. Although there was no difference in capillary density between the two groups, the authors concluded that the increased blood flow contributed to the enhanced skeletal muscle glucose uptake in the athletes by allowing the opening of more capillaries during hyperinsulinemia (19).

It is important to note that muscle glucose uptake is equal to the product of the arteriovenous glucose difference and the rate of glucose delivery or muscle blood flow. Although it is generally accepted that insulin will accelerate blood glucose extraction by muscle, recent evidence

suggests that it has hemodynamic effects to increase both total blood flow (20) and capillary recruitment (21). Using new approaches, we have recently shown that insulin at physiological doses increases muscle capillary recruitment in rats (21) and humans (22). Furthermore, when capillary recruitment is blocked by pharmacological means (23) or tumor necrosis factor-α (TNF-α) administration (24), an acute state of insulin resistance occurs, causing as much as 50% of insulin-mediated glucose uptake to be impaired. Thus, in the present study, we have exposed formerly sedentary laboratory rats to a voluntary exercise-training program over a 14-day period and assessed their responsiveness to insulin using the hyperinsulinemic-euglycemic clamp, with particular focus on hemodynamic parameters.

RESEARCH DESIGN AND METHODS

Animals. Male hooded Wistar rats weighing 245 ± 3 g were raised on a commercial diet (Gibsons, Hobart, Australia) containing 21.4% protein, 4.6% lipid, 68% carbohydrate, and 6% crude fiber, with vitamins and minerals added, together with water ad libitum. Rats were individually housed at a constant temperature of $21 \pm 1°C$ in a 12 h light/12 h dark cycle in either standard 18×30 cm cages or exercise cages, where they had the option to run. The exercise cages (1 m diameter and 12 cm wide) rotated on a horizontal axis as the rat ran; an odometer monitored the number of rotations. All procedures adopted and experiments undertaken were approved by the University of Tasmania Animal Ethics Committee.

Surgery. Rats were anesthetized using Nembutal (50 mg/kg body wt) and had polyethylene cannulas (PE-50; Intramedic) surgically implanted into the carotid artery for arterial sampling and measurement of blood pressure (pressure transducer Transpac IV; Abbott Critical Systems) and into both jugular veins for continuous administration of anesthetic and other intravenous infusions. A tracheotomy tube was inserted, and the animal was allowed to spontaneously breathe room air throughout the course of the experiment. Small incisions (1.5 cm) were made in the skin overlaying the femoral vessels of both legs, and the femoral artery was separated from the femoral vein and saphenous nerve. The epigastric vessels were then ligated, and an ultrasonic flow probe (VB series 0.5 mm; Transonic Systems) was positioned around the femoral artery of the right leg just distal to the rectus abdominis muscle. The cavity in the leg surrounding the flow probe was filled with lubricating jelly (H-R; Mohawk Medical Supply, Utica, NY) to provide acoustic coupling to the probe. The probe was then connected to the flow meter (Model T106 ultrasonic volume flow meter; Transonic Systems), which was interfaced with an IBM-compatible PC computer that acquired the data (at a sampling frequency of 100 Hz) for femoral blood flow (FBF), heart rate, and blood pressure using Windaq data acquisition software (Dataq Instruments). The surgical procedure generally lasted ~30 min, and then the animals were maintained under anesthesia for the duration of the experiment using a continual infusion of Nembutal ($0.3–0.6$ mg $\cdot$ min^{-1} $\cdot$ kg^{-1}) via the left jugular cannula. The femoral vein of the left leg was used for venous sampling, which was performed using an insulin syringe with an attached 29G needle (Becton Dickinson). A duplicate venous sample (V) was taken only on completion of the experiment (120 min) to prevent alteration of the blood flow from the hindlimb due to sampling and to minimize the effects of blood loss. The body temperature was maintained using a water-jacketed platform and a heating lamp positioned above the rat.

Experimental procedures. Surgery was followed by a 60-min equilibration period to allow leg blood flow and blood pressure to become stable and constant. Rats were then allocated into either the control (saline) or euglycemic insulin clamp group (including subgrouping of sedentary and 14-day exercise-trained rats, $n = 8$ in each group). Saline infusion in the control groups was matched to the volumes of insulin (Humulin R; Eli Lilly, Indianapolis, IN) and glucose infused in the euglycemic insulin clamp. Because 1-MX (Sigma Aldrich) clearance was very rapid, it was necessary to partially inhibit the activity of xanthine oxidase (21), particularly in nonmuscle tissues. To do this, an injection of a specific xanthine-oxidase inhibitor, allopurinol (10 μmol/kg) (25), was administered as a bolus dose 5 min before commencing the 1-methylxanthine (1-MX) infusion (0.4 mg $\cdot$ min^{-1} $\cdot$ kg^{-1}). This allowed constant arterial concentrations of 1-MX to be maintained throughout the experiment.

The total blood volume withdrawn from the animals before the final arterial and venous samples did not exceed 1.5 ml and was easily compensated by the volume of fluid infused.

Duplicate arterial (A) and venous (V) samples (300 μl) were taken at the end of the experiment (180 min) and placed on ice. These blood samples were immediately centrifuged, and 100 μl plasma was mixed with 20 μl of 2 mol/l perchloric acid. The perchloric acid–treated samples were then stored at $-20°C$ until they were assayed for 1-MX. The rest of the plasma was used for plasma glucose and plasma insulin analysis.

Analytical methods. A glucose analyser (Model 2300 Stat Plus; Yellow Springs Instruments) was used to determine whole-blood glucose (by the glucose oxidase method) during the insulin clamp. A blood sample of 25 μl was required for each determination. Insulin levels at the beginning and end of the euglycemic insulin clamp were determined from arterial plasma samples by enzyme-linked immunosorbent assay (Mercodia AB, Uppsala Sweden) using human and rat insulin standards where appropriate. Perchloric acid–treated plasma samples were centrifuged for 10 min, and the supernatants were used to determine 1-MX, allopurinol, and oxypurinol by reversephase high-performance liquid chromatography (HPLC), as previously described (21,23).

Xanthine oxidase was assayed in vastus and combined samples of the soleus, gastrocnemius, and plantaris muscles. These muscles were frozen in liquid N_2 at the end of the experiment, powdered, and stored at $-70°C$ until assayed. Muscle powder (400 mg) was homogenized in 2 ml buffer (50 mmol/l Na_2HPO_4, 0.1 mmol/l Na_2EDTA, 40 mmol/l dithiothreitol, and 0.5 mg/ml trypsin inhibitor; pH 7.4) and then centrifuged for 30 min at $50,000g$. The supernatant was passed through a PD-10 desalting column (Amersham Pharmacia Biotech), and the protein fractions were collected. A sample of the protein fraction (100 μl) was added to the assay mix (0.9 ml), which consisted of buffer (as described above) and 100 μmol/l xanthine, the substrate for xanthine oxidase, and was incubated at $37°C$ for 30 min. The reaction was stopped by adding 250 μl assay mix to 50 μl of 2 mol/l perchloric acid. The amount of uric acid formed from xanthine was determined by HPLC using a Luna $5μ$ C8 column (Phenomenex) with isocratic separation in 50 mmol/l $NH_4H_2PO_4$ (pH 3.5) buffer at 1.2 ml/min. Protein amounts were determined using a Bradford protein assay kit (Bio-Rad Laboratories). Activity was expressed as pmol $\cdot$ min^{-1} $\cdot$ mg^{-1} protein.

Capillary density. Capillary endothelial cells were identified by the binding of *Griffonia* (*Bandeiraea*) *simplicifolia* lectin (GSL)-1 (Vector, Burlingame, CA). Soleus, plantaris, and extensor digitorum longus muscles were dissected from sedentary or exercise-trained rats, cut into 2-mm transverse blocks, and fixed in Bouins fixative (10% formaldehyde in saturated picric acid solution with 5% glacial acetic acid). Tissues were processed to paraffin as previously described (26), with four blocks from each muscle mounted in each paraffin block. Paraffin sections (6 μm) were mounted on silanized slides (Dako, Carpintaria, CA) and dewaxed. Sections were treated for 6 min with 125 μg/ml pronase E in 50 mmol/l Tris buffer, pH 7.6, rinsed twice in Tris buffer with 0.2% glycine, and then rinsed once in Tris buffer. After a 20-min incubation with peroxidase-blocking reagent (Dako), sections were transferred to Tris buffer with 0.9% NaCl and additional salts (1 mmol/l $CaCl_2$, $MnCl_2$, and $MgCl_2$). Sections were incubated for 2 h with GSL-1 in Tris buffer with 0.9% NaCl and additional salts and 0.01% Nonidet P-40 (Sigma, St. Louis, MO). After being rinsed in Tris buffer with 0.9% NaCl with salts, sections were incubated with goat anti–GSL-1 (Vector) primary antibody (1 μg/ml). The biotinylated secondary antibody and peroxidase binding were performed using a (goat) Vectastain ABC kit (Vector) as previously described (26). Four sections per muscle per rat ($n = 4$) were photographed (magnification $\times 60$), and the number of capillaries surrounding each muscle fiber were counted.

Data analysis. All data are expressed as means $\pm$ SE. Mean FBF, heart rate, and arterial blood pressure were calculated from 5-s subsamples of the data, representing ~500 flow and pressure measurements, every 15 min. Vascular resistance in the hindleg was calculated as the mean arterial blood pressure (in millimeters of mercury) divided by FBF (in milliliters per minute) and expressed as resistance units. Glucose uptake in the hindlimb was calculated from arteriovenous glucose difference and multiplied by FBF and expressed as micromoles per minute. The 1-MX disappearance was calculated from arteriovenous plasma 1-MX difference and multiplied by FBF (corrected for the volume accessible to 1-MX, 0.871, which was determined from plasma concentrations obtained after standard 1-MX was added to whole rat blood) and expressed as nanomoles per minute.

Statistical analysis. To ascertain differences between treatment groups at the end of the experiment (120 min), one-way analysis of variance was used. When a significant difference ($P < 0.05$) was found, Dunnett's test was used to determine which times were significantly different from saline control (used for FBF, arterial blood pressure, femoral vascular resistance, arterial glucose and 1-MX, hindleg glucose extraction and uptake, and hindleg 1-MX extraction and disappearance). Pairwise comparisons were made using Student-New-
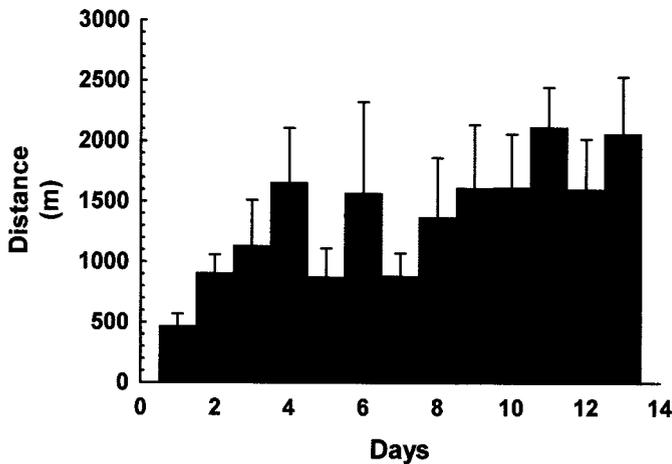
FIG. 1. Average distance run each day by the rats on the exercise-training program. Values are means ± SE for $n = 8$.

FIG. 2. Effect of exercise training for 14 days on heart and muscle weight. Values are means ± SE for $n = 8$ in both the sedentary (□) and exercise-trained (■) groups. *Significantly different ($P < 0.05$) from sedentary animals.

man-Keuls method. An unpaired Student's t test was used to determine whether there was a significant difference ($P < 0.05$) between the glucose infusion rates (GIRs) at the conclusion of the experiments. All tests were performed using the SigmaStat statistical program (Jandel Software).

RESULTS

Effects of exercise training. Saline-infused rats weighed 245 ± 3 g, insulin clamp–treated animals under sedentary conditions weighed 235 ± 6 g, and rats undergoing 14 days of exercise training weighed 253 ± 8 g. Figure 1 shows the average distance run each day over the 14-day training period. Running was tentative at the start of the program. Thus, the average was only 471 m for the first day. This increased steadily over the next 3 days and reached a plateau at ~1,650 m. At the end of the 14-day period, the rats had run ~18 km.

Muscle weight. Figure 2 shows heart and lower-leg muscle weights before and after the 14-day exercise-training period. A significant ($P < 0.05$) but small (<20%) increase was noted for the soleus muscle.

FBF. Figure 3 shows the changes in FBF, blood pressure, and calculated hindleg vascular resistance after saline or insulin infusions for sedentary rats or rats trained for 14 days. There were no significant changes observed in blood pressure. For sedentary animals, insulin increased FBF from 0.93 ± 0.09 to 1.49 ± 0.13 ml/min. Exercise training for 14 days tended to augment the insulin-mediated increase in FBF, but this was not significant when compared with insulin alone in sedentary animals. Femoral vascular resistance showed a similar nonsignificant trend.

Glucose metabolism during the insulin clamp. Before the infusion of either saline or insulin, initial arterial glucose concentrations did not differ between sedentary (4.3 ± 0.12 mmol/l) and exercise-trained (4.5 ± 0.12 mmol/l) animals. Figure 4 shows the plateau values for the GIRs. For insulin clamp–treated sedentary rats, the GIR was 123.7 ± 2.7 μmol · min^{-1} · kg^{-1}. This was significantly increased by exercise training for 14 days to 154.2 ± 4.2 μmol · min^{-1} · kg^{-1}. Hindleg glucose extraction was increased by insulin alone and marginally increased by exercise training (data not shown). Because of this and the tendency for the insulin-mediated increase in FBF to be augmented by exercise training (Fig. 3), there was a significant increase in hindleg glucose uptake due to
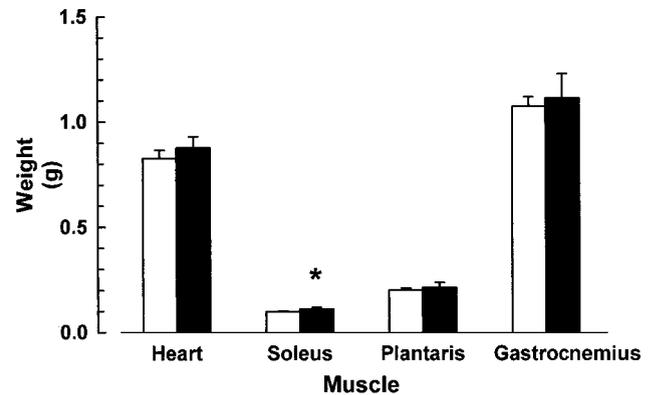
insulin; the increment due to insulin increased from 0.44 to 0.85 μmol/min as a result of exercise training (Fig. 4).

1-MX metabolism. No significant difference was found in arterial plasma concentrations of 1-MX between the saline and insulin groups, and there was no difference as a result of exercise training. At the end of the experiment (180 min), arterial 1-MX concentrations were 17.7 ± 0.8 μmol/l in the saline-sedentary group and 17.6 ± 0.9 μmol/l in the insulin-sedentary group. Although there was a tendency for arterial 1-MX to be higher in the exercise-trained groups at the end of the insulin clamp, it was not significant (Fig. 5). Extraction of 1-MX by the hindleg was ~30% and unaffected by insulin in the sedentary animals. Those that were exercise-trained for 14 days showed a tendency for insulin to increase 1-MX extraction, but this was not significant. Hindleg 1-MX disappearance, the product of 1-MX extraction, and FBF showed a significant increase due to insulin in sedentary rats, and this was further increased due to training. Figure 5 shows that the increment of 1-MX disappearance increased with insulin treatment, from 3.4 to 5.5 nmol/min, representing an increase of ~62%.

Plasma insulin concentrations. There was no significant difference in initial arterial plasma insulin levels (before insulin or saline infusion) between the sedentary (239 ± 27 pmol/l) and exercise-trained (259 ± 44 pmol/l) groups. Insulin infusion resulted in a significant ($P < 0.001$) increase compared with saline infusion, but there was still no significant difference between the sedentary and exercised-trained groups after either saline (sedentary, 368 ± 44 pmol/l; exercise-trained, 325 ± 82 pmol/l) or insulin infusion (sedentary, 1,993 ± 299 pmol/l; exercise-trained, 1,962 ± 251 pmol/l).

Xanthine oxidase. There was no significant difference in maximal catalytic activity of muscle xanthine oxidase between sedentary (128 ± 13 pmol · min^{-1} · mg protein^{-1}; $n = 12$) and exercise-trained animals (143 ± 10 pmol · min^{-1} · mg protein^{-1}; $n = 13$).

Capillary denisty. There was no significant difference in capillary density, expressed as capillary contacts per fiber, between sedentary and exercise-trained rats in soleus (6.74 ± 0.26 vs. 6.50 ± 0.08), plantaris (5.48 ± 0.16 vs. 5.35 ± 0.21), or extensor digitorum longus (5.15 ± 0.07 vs. 5.11 ± 0.09) muscles.
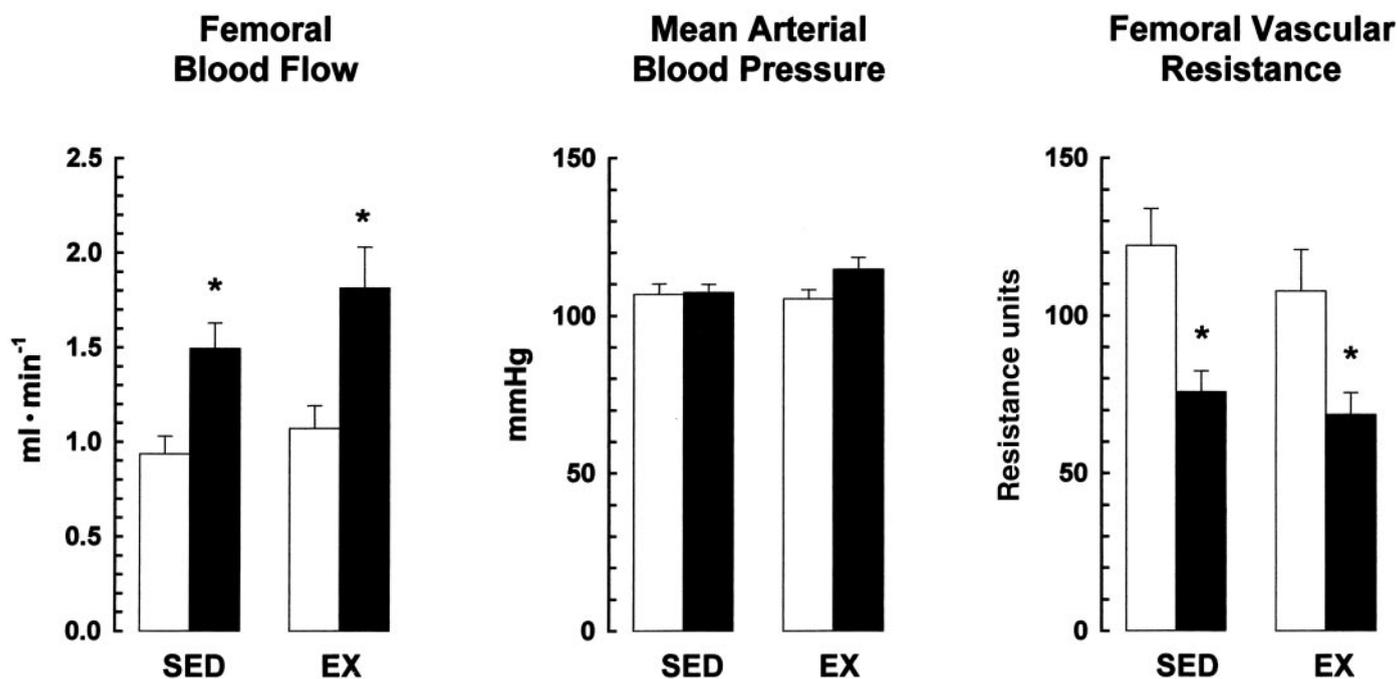
Femoral Blood Flow

Mean Arterial Blood Pressure

Femoral Vascular Resistance

FIG. 3. Systemic and hindleg changes in hemodynamic factors of saline (□) and insulin clamp–treated (■) rats under sedentary (SED) or 14-day exercise-training (EX) programs. Data were collected at 120 min after the start of insulin or saline infusion. Values are means ± SE for $n = 8$ in each group. *Significantly different ($P < 0.05$) from saline.

DISCUSSION

The present study shows that exercise training improves insulin-mediated glucose uptake by rat hindleg muscle under hyperinsulinemic-euglycemic clamp conditions. This was manifested as a 93% increase in insulin-mediated glucose uptake across the leg when compared with sedentary animals. Whole-body glucose clearance was also improved as a result of exercise training, as reflected by an increased GIR (24%) to maintain euglycemia. These changes have been noted by others as typical adaptations
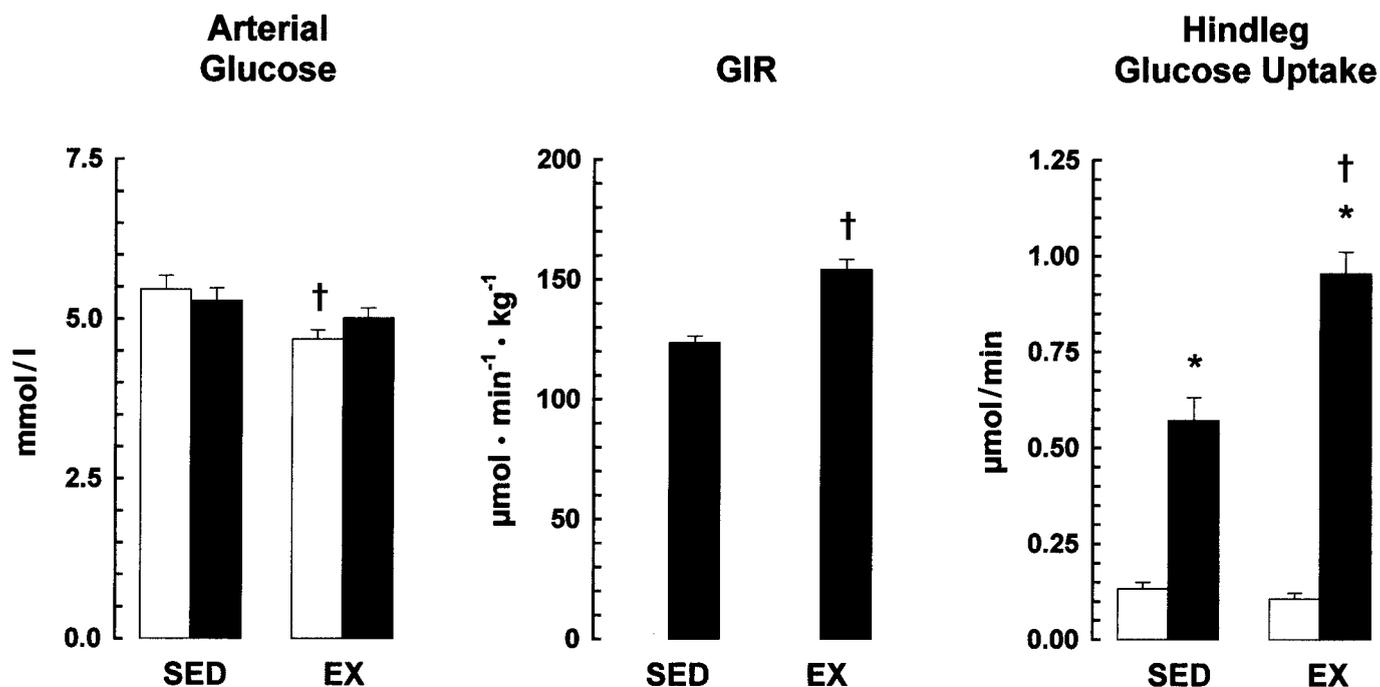
Arterial Glucose

GIR

Hindleg Glucose Uptake

FIG. 4. Systemic and hindleg glucose values of saline (□) and insulin clamp–treated (■) rats under sedentary (SED) or 14-day exercise-training (EX) programs. Values are means ± SE for $n = 8$ in each group. *Significantly different ($P < 0.05$) from corresponding saline-treatment control; †significantly different from SED animals.

FIG. 5. Systemic and hindleg 1-MX values of saline (□) and insulin clamp–treated (■) rats under sedentary (SED) or 14-day exercise-training (EX) programs. Values are means ± SE for $n = 8$ in each group. *Significantly different ($P < 0.05$) from corresponding saline-treatment control; †significantly different from SED animals.
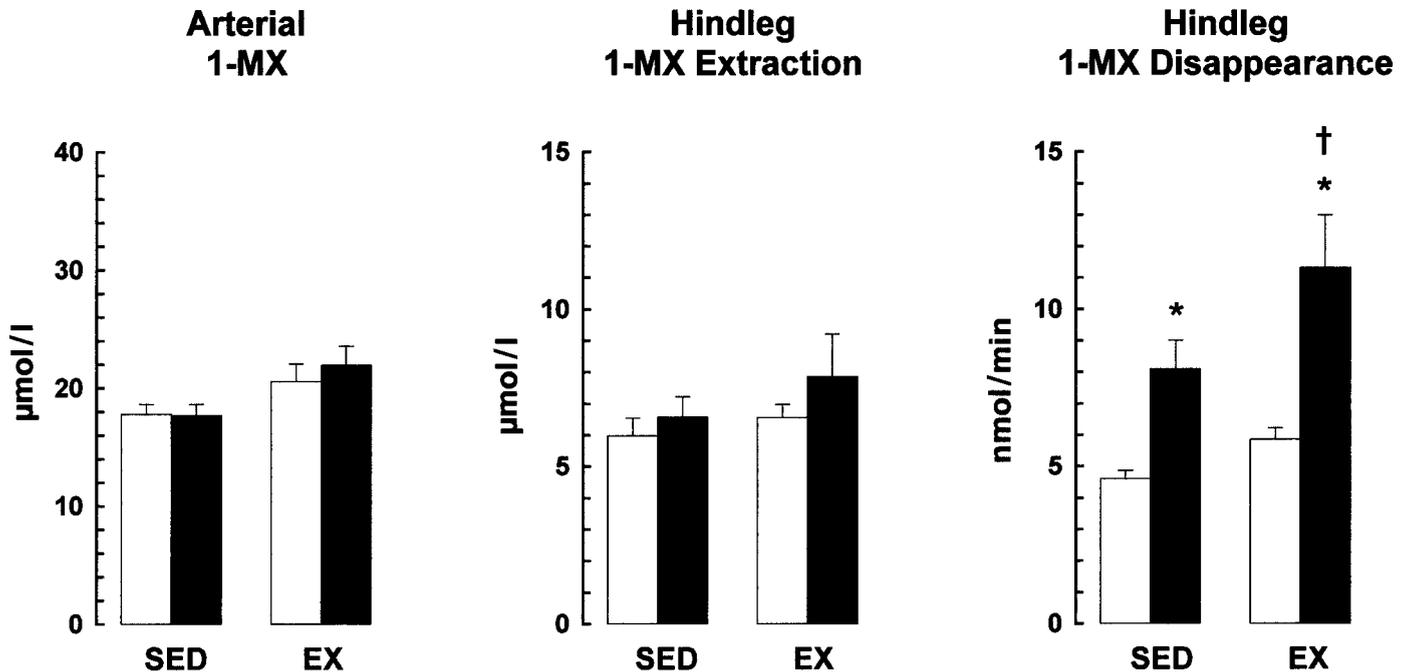
to exercise training in both animal models and humans (16,17). More importantly, in relation to the present study, there was a marked enhancement in insulin-mediated capillary recruitment, as reflected by increased metabolism of 1-MX.

This represents the first report in which exercise training has led to enhanced insulin-mediated effects on leg muscle capillary recruitment. However, a key issue is whether the enhanced capillary recruitment due to insulin is responsible for the enhanced insulin-mediated leg glucose uptake. Total FBF, capillary recruitment, or a combination of the two may increase insulin and glucose access and thereby enhance insulin-mediated glucose uptake. Although there were significant differences in FBF between groups, with the insulin-treated groups having significantly higher FBF than the saline-treated groups, there was no significant difference in FBF between the sedentary and exercise-trained groups. The power of the one-way analysis of variance test with an α value of 0.05 was 0.95, indicating that the possibility of a type II error is small. Accordingly, it is unlikely that differences in FBF can account for the increased insulin-mediated glucose uptake in these rats. In addition, from our previous studies in which FBF was deliberately increased by epinephrine infusion, there was no accompanying increase in either 1-MX metabolism or leg glucose uptake (21). Furthermore, time-course data at physiologic insulin levels ($3 \, \text{mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, which give plasma levels of 500 pmol/l) indicates that capillary recruitment is an early event that possibly occurs before maximum glucose uptake has been reached (27). Also, pharmacological attempts to block capillary recruitment by redirecting blood flow to the non-nutritive route (23) markedly inhibited insulin-mediated glucose uptake by the leg. However, in the present study at $10 \, \text{mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, both FBF and capillary recruitment were

increased in the sedentary and exercise-trained animals; therefore, it is possible that the increased blood flow that resulted from increasing substrate and insulin delivery to muscle under conditions of complete capillary recruitment further augmented glucose uptake, particularly in the exercise-trained rats.

A potential weakness of the present study is that 1-MX metabolism is not necessarily elucidated by measuring changes in capillary exposure and that the increase that occurred during the hyperinsulinemic-euglycemic clamp is not the result of capillary recruitment. The 1-MX method was developed from isolated pump perfused rat hindlimb studies in which the complexities of in vivo homeostatic processes could be avoided and total limb flow could be controlled. When perfused at constant flow, vasoconstrictors act in the perfused hindlimb to alter the proportion of nutritive to non-nutritive flow (28). A high proportion of nutritive flow was found to correspond with a high rate of metabolism and an enhanced aerobic tension development during electrical stimulation (28). Similarly, a high proportion of non-nutritive flow depressed metabolism and inhibited aerobic muscle contraction, resulting in an acute state of insulin resistance in which insulin and glucose had reduced access to the muscle fibers (28). Others have shown that xanthine oxidase is predominantly located in capillary endothelial cells (29), and on this basis, we conducted experiments to see whether the metabolism of infused 1-MX, targeted for the capillary endothelial cells, changed in proportion to nutritive (capillary) flow. We found that when flow was predominantly non-nutritive, metabolism of 1-MX was markedly reduced (30), and when flow was predominantly nutritive, as with exercise, metabolism was increased (31). On this basis, 1-MX metabolism was used to assess changes in capillary recruitment due to insulin under normal conditions (21), when non-nutritive

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flow predominated (23), and in the presence of TNF-α (24). Two other methods that were based on principles that differ from 1-MX metabolism have recently been used and have given results similar to 1-MX. For example, laser Doppler flowmetry (LDF) was used to monitor muscle microvascular blood flow during the hyperinsulinemic-euglycemic clamp in rats, where it was found that the LDF signal increased at 30 min, preceding the increase in FBF (32). Our conclusion that capillary recruitment had occurred was based on a companion study using the perfused rat hindlimb in which the LDF probes were characterized and essentially found to be measuring changes in muscle nutritive flow, albeit capillary recruitment (33). A second technique, based on yet another principle, is an adaptation of contrast-enhanced ultrasound (CEU) imaging. This technique is performed by ultrasound imaging of tissue during simultaneous intravenous administration of gas-filled microbubble contrast agents. These microbubbles possess a similar microvascular rheology as erythrocytes (34) and generate an acoustic signal as they pass through the microcirculation imaged by the ultrasound beam, thereby producing tissue opacification. At ultrasound powers conventionally used, microbubbles are destroyed by the imaging pulse of ultrasound; therefore, CEU must be performed with long time intervals between frames. When these intervals are long enough to allow capillaries to completely refill after each pulse, and a steady-state concentration of microbubbles in blood is achieved by a continuous intravenous infusion, the microbubble signal in tissue reflects the capillary blood volume in tissue (35). Changes in capillary blood volume in response to physiological insulin have recently been assessed using CEU in the skeletal muscle of the rat hindlimb (27). Compared with baseline values, saline infusion resulted in little change in capillary blood volume, whereas marked increases in capillary blood volume occurred during euglycemic insulin clamp ($3 \text{ mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$). The changes correlated well with the 1-MX method and also showed that significant changes in capillary recruitment had occurred at physiological doses of insulin without changes in total blood flow (27).

Although our findings from a number of studies (21,23,24) imply that insulin-mediated capillary recruitment is in part responsible for insulin-mediated glucose uptake by the leg, it is possible that the hemodynamic changes induced by insulin are secondary to increased glucose uptake by the muscle cells. In this scenario, we would foresee that muscle glucose metabolism produces a vasodilator that acts locally to recruit capillary flow. Thus, enhanced insulin-mediated capillary recruitment after exercise training would then be secondary to changes occurring to glucose metabolism that are intrinsic to the muscle cells. In this regard, others have shown that exercise training resulted in increased expression of a number of proteins and structures so that the muscles are now better adapted to support increased aerobic capacity and the energy required for more strenuous exercise. Presumably, these adaptations would also allow increased production of vasodilatory factor(s) to enhance capillary recruitment and possibly total flow.

Changes independent of adaptations within the skeletal muscle cells that account for enhanced hemodynamic parameters are not well-defined because insulin's hemodynamic effects were recently recognized. In the present study, the exercise regimen was voluntary and thus mild-to-moderate. Xanthine-oxidase activity in muscle homogenates did not change as a result of exercise training, and there was no increase in capillary density in a range of muscles. Consistent with this, hindleg disappearance of 1-MX in the absence of added insulin (i.e., saline controls) was not greater in trained versus untrained animals (Fig. 5). In the absence of changes in capillary density, the present findings of enhanced insulin-mediated capillary recruitment and leg glucose uptake would imply that insulin signaling leading to vasodilator production is up-regulated.

In summary, exercise training was found to increase insulin-mediated glucose uptake by the hindlimb in association with enhanced capillary recruitment, as reflected by increased 1-MX metabolism. We conclude that the enhanced capillary recruitment is in part responsible for the increased glucose uptake by muscle.

ACKNOWLEDGMENTS

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