Original Article

Contraction Stimulates Nitric Oxide–Independent Microvascular Recruitment and Increases Muscle Insulin Uptake

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We examined whether contraction-induced muscle microvascular recruitment would expand the surface area for insulin and nutrient exchange and thereby contribute to insulin-mediated glucose disposal. We measured in vivo rat hindlimb microvascular blood volume (MBV) using contrast ultrasound and femoral blood flow (FBF) using Doppler ultrasound in response to a stimulation frequency range. Ten minutes of 0.1-Hz isometric contraction more than doubled MBV ($P < 0.05$; $n = 6$) without affecting FBF ($n = 7$), whereas frequencies >0.5 Hz increased both. Specific inhibition of nitric oxide (NO) synthase with N^{ω}-L-nitro-arginine-methyl ester ($n = 5$) significantly elevated mean arterial pressure by ~30 mmHg but had no effect on basal FBF or MBV. We next examined whether selectively elevating MBV without increasing FBF (0.1-Hz contractions) increased muscle uptake of albumin-bound Evans blue dye (EBD). Stimulation at 0.1 Hz (10 min) elicited more than twofold increases in EBD content (micrograms EBD per gram dry tissue) in stimulated versus contralateral muscle ($n = 8$; 52.2 ± 3.8 vs. 20 ± 2.5, respectively; $P < 0.001$). We then measured muscle uptake of EBD and ^{125}I-labeled insulin (dpm per gram dry tissue) with 0.1-Hz stimulation ($n = 6$). Uptake of EBD (19.1 ± 3.8 vs. 9.9 ± 1; $P < 0.05$) and ^{125}I-insulin (5,300 ± 800 vs. 4,244 ± 903; $P < 0.05$) was greater in stimulated muscle versus control. Low-frequency contraction increases muscle MBV by a NO-independent pathway and facilitates muscle uptake of albumin and insulin in the absence of blood flow increases. This microvascular response may, in part, explain enhanced insulin action in exercising skeletal muscle. *Diabetes* 56:2194–2200, 2007

Exercise reduces the risk of developing insulin resistance and type 2 diabetes and improves insulin sensitivity in insulin-resistant populations (1,2) through activation of a number of metabolic pathways in skeletal muscle. These include AMP-activated kinase (3,4), mitogen-activated protein kinase (5,6), Akt (7,8), and the p70^{s6k} (9) pathways. These changes potentiate the effect of insulin to increase skeletal muscle glucose transport (10–13) and uptake (7,14), to increase fatty acid oxidation (15,16), and to increase mitochondrial activity (17,18). In healthy human volunteers, performing low-grade exercise during a euglycemic insulin clamp increases whole-body and leg glucose uptake to a greater extent than either insulin or exercise alone or even the sum of their individual effects (19).

Although exercise clearly enhances insulin action on skeletal myocytes, studies suggest that the rate-limiting step to the metabolic actions of insulin in skeletal muscle is its transport from the vasculature to the muscle interstitium (20,21). The capillary endothelium is a physical barrier that macromolecules must cross to access muscle tissue. It is thought that insulin is transported in one or more ways, including passive diffusion (22,23), vesicular movement (24,25), and receptor-mediated transcytosis (26). Regardless of the mechanism of the transendothelial transport (TET) of insulin, it remains unknown whether exercise enhances the uptake of insulin to muscle, where it activates multiple metabolic pathways.

Insulin increases muscle blood flow and induces microvascular recruitment in both humans (27–29) and animals (30–33). Both processes are inhibited by nitric oxide (NO) synthase (NOS) blockade in rats (33). These insulin-induced increases in microvascular blood volume (MBV) expand endothelial surface area and may thereby facilitate the transfer of nutrients and macromolecules (like insulin) from the vasculature to muscle interstitium. Whether exercise recruits skeletal muscle microvasculature by a mechanism similar to insulin is unknown. Dawson et al. (34) examined microvascular recruitment in skeletal muscle in response to insulin or 2-Hz isometric skeletal muscle contraction and concluded that each can increase both MBV and total hindlimb femoral blood flow (FBF). Subsequently, Wheatley et al. (35) observed that in the obese Zucker rat, insulin-mediated recruitment is blunted, but 2-Hz hindlimb contraction still effectively recruits microvasculature. This suggests that exercise might recruit microvasculature via a mechanism that is distinct from that of insulin.

The 2-Hz stimulation frequency used in previous studies (34,35) increased FBF in addition to recruiting hindlimb microvasculature. In this study, we hypothesized (as had been suggested by Honig et al. [36] from analysis of tissue sections) that low-frequency contraction effectively increases skeletal muscle MBV without significantly increasing FBF. This would allow us to address the functional

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Received for publication 5 January 2007 and accepted in revised form 1 June 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 5 January 2007. DOI: 10.2337/db07-0020.

AV, arteriovenous; CEU, contrast-enhanced ultrasound; EBD, Evans blue dye; FBF, femoral blood flow; L-NAME, N^{ω}-L-nitro-arginine-methyl ester; MAP, mean arterial pressure; MBV, microvascular blood volume; MFV, microvascular flow velocity; NOS, nitric oxide synthase; PECAM-1, platelet-endothelial adhesion molecule-1; TET, transendothelial transport.

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consequences of microvascular recruitment on the transfer of macromolecules from plasma into muscle tissue in vivo. We did this first using Evans blue dye (EBD), a tetrasodium diazo salt (molecular weight 980 Da) that rapidly and irreversibly (37) binds to albumin in a 10:1 molar ratio (38), and subsequently using I^{125}-insulin as a tracer. In addition, we examined whether contraction-induced recruitment, changes in FBF, or muscle EBD uptake was affected by infusion of the NOS inhibitor N^{ω}-L-nitro-arginine-methyl ester (L-NAME).

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats (200–250 g) were obtained from Charles River Laboratories (Wilmington, MA), housed at 22 ± 2°C with a 12:12-h light:dark cycle, and allowed free access to water and standard chow diet. Rats were fasted overnight before all experiments. The experimental protocols followed were approved by the Animal Care and Use Committee of the University of Virginia.

Surgical procedures. Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50–55 mg/kg body wt) and placed on a surgical platform. Body temperature was maintained at 38°C with a heating pad. The jugular vein and carotid artery were cannulated with polyethylene tubing (PE-50; Intramedic) for intravenous infusions, arterial blood sampling, and monitoring of mean arterial pressure (MAP). The animals breathed spontaneously through a tracheotomy tube for the duration of the experiment. For experiments where total FBF was measured, the femoral vessels in the right hindlimb were exposed as described previously (29,35), and an ultrasound flow probe (VB series 0.5 mm; Transonic Systems) was positioned over the femoral artery. The flow probe was interfaced through a flow meter to an IBM-compatible computer. FBF and MAP were continuously acquired using Windaq software (Dataq Instruments, Akron, OH).

Protocol 1: hindlimb microvascular recruitment and glucose uptake in response to electrical stimulation. Rats were given either a continuous saline infusion (n = 6) or a continuous infusion of the NOS inhibitor (n = 5) L-NAME (50 $\mu g \cdot min^{-1} \cdot kg^{-1}$; Sigma Chemicals, St. Louis, MO). We previously observed that this L-NAME dose raises MAP 20–30 mmHg above baseline without affecting heart rate and completely inhibits insulin-mediated increases in both FBF and MBV (33). All animals received a continuous infusion (20 µl/min) of Definity microbubbles (Bristol-Myers Squibb Medical Imaging, North Billerica, MA) that began 10 min before and continued throughout the stimulation protocol. MBV was measured continuously using contrast-enhanced ultrasound (CEU) in response to contraction frequency. In brief, high-power, pulse-inversion ultrasound (HDI-5000; Philips Ultrasound) was used to image the proximal adductor muscle group (adductor magnus and semimembranosus) of the right hindlimb as previously described (33,39). Two metal electrodes were placed in the adductor tendons of the right hindlimb, and the limb was secured in place. The left hindlimb served as the sham control. Muscles were stimulated with a 2-V square-wave pulse for 0.5 ms, resulting in isometric contraction for a very brief duration (Grass S88 Pulse Generator; Astro-Med, West Warwick, RI). The electrical stimulation frequency was adjusted in a stepwise manner every 10 min over a range of seven frequencies (0, 0.05, 0.1, 0.2, 0.5, 1, and 2 Hz). The epigastric vein was cannulated to sample blood from the femoral vein of the stimulated leg. Arterial and venous blood samples were drawn before and at the end of each bout of stimulation. Whole-blood glucose was measured in duplicate using a YSI analyzer (Yellow Springs Instruments, Yellow Springs, OH). Hindlimb glucose uptake was calculated as the arteriovenous (AV) glucose concentration (millimoles per liter) difference multiplied by FBF (milliliters per minute).

Protocol 2: muscle EBD uptake in response to low-frequency contraction. Rats either received a continuous infusion of saline (n = 8) or L-NAME (n = 4) as described for protocol 1. A bolus of EBD (25 mg/kg body wt) was given intravenously, via jugular vein, and allowed to circulate for 5 min before the starting low-frequency electrical stimulation (0.1 Hz, 2 V, 0.5 ms) of the right hindlimb. This frequency was selected because it was found in protocol 1 to increase skeletal muscle MBV without changing femoral artery blood flow. After 10 min of low-frequency stimulation, the animal was killed and perfused with 120 cc of cold, heparinized saline retrograde via the carotid artery to clear residual intravascular dye. Muscles (gastrocnemius, soleus, and gracilis) were sampled from both hindlimbs, and EBD content was measured spectrophotometrically (40) and expressed as micrograms dye per gram dry tissue.

Protocol 3: muscle ^{125}I-labeled insulin and EBD muscle content in response to low-frequency contraction. Rats received a continuous infusion of saline and a bolus of EBD as described for protocol 2 (n = 6). Low-frequency stimulation (0.1 Hz, 2 V, 0.5 ms) began 5 min after the EBD bolus for a total duration of 10 min as described for protocols 1 and 2. Five minutes after starting the stimulation, rats were given a 1.5-µCi bolus of ^{125}I-insulin (Amersham Biosciences, Buckinghamshire UK). This mono-iodinated species reportedly has insulin receptor–binding characteristics similar to native insulin. This tracer amount of insulin does not increase systemic insulin concentrations and therefore will not induce muscle microvascular responses. The experiment was terminated as described in protocol 2 followed by a systemic ice-cold flush retrograde via the carotid artery to clear residual vascular EBD and ^{125}I-insulin. Gastrocnemius, soleus, and gracilis muscles were dissected from the stimulated and the control contralateral hindlimb. Protein-bound 125-iodine in blood and muscle samples was precipitated using 10% trichloroacetic acid, and radioactivity was measured in a gamma counter and expressed as dpm per gram dry tissue. EBD muscle content was measured as described in protocol 2.

Immunohistochemistry and confocal imaging. Samples of tibialis muscles from protocol 2 animals were snap frozen in isopentane cooled in liquid nitrogen. Frozen sections (7 µm thick) were fixed in cold acetone/methanol, and an antibody against platelet-endothelial adhesion molecule-1 (PECAM-1) (SC-1506; Santa Cruz Biosciences, Santa Cruz CA) immunocytochemically stained the vasculature. When exposed to a green laser (568 nm), EBD fluoresces red. Therefore a green fluorescent secondary antibody (AlexaFluor 488; Santa Cruz Biosciences) was used to visualize PECAM-1 at 488 nm. Immunocytochemical labeling was examined simultaneously using a two-color BX50 WI confocal microscope (Olympus, Tokyo, Japan) equipped with krypton and argon laser. The samples were scanned at magnification ×60, and a series of optical sections (thickness <0.1 µm) was acquired from the top to the bottom at a resolution of 1,024 × 1,024 pixels and stored in 24-bit image format.

Statistical analysis. Data are presented as means ± SE. Two-way repeated-measures ANOVA was used to assess the effect of contraction frequency and L-NAME on contraction-mediated changes in microvascular volume. When a significant difference of $P < 0.05$ was found, pairwise comparisons were made using Student-Newman-Keuls test. Student's paired t test was used for statistical comparisons within each treatment group for protocols 2 and 3. Statistical significance was considered at $P < 0.05$.

RESULTS

Protocol 1: low-frequency electrical stimulation recruits capillaries by an NO-independent pathway without increasing FBF. In response to 10 min of isometric contraction at each of seven different frequencies, we observed a frequency-dependent increase in FBF in the saline-infused animals (n = 6). FBF was 0.53 ± 0.03 ml/min at baseline and did not change significantly (0.59 ± 0.05 ml/min at 0.05 Hz; 0.64 ± 0.06 ml/min at 0.1 Hz) at stimulation frequencies below 0.2 Hz in saline-infused animals (Fig. 1*A*). However, FBF increased ~threefold between 0.2 and 2 Hz (0.77 ± 0.06 and 2.4 ± 0.26 ml/min, respectively). There was likewise a frequency-dependent increase in FBF in the NOS-inhibited group (n = 5) despite the fact that L-NAME was infused beginning 30 min before and continuing throughout the period of electrical stimulation. MAP in the L-NAME group was ~30 mmHg above (122 vs. 92) the saline group, indicating that L-NAME infusion effectively inhibited NOS (Fig. 1*B*). With this, there was a small (26 beats/min) decline in heart rate over 45 min that was not statistically significant (not shown). In these animals as well, FBF only rose significantly above baseline at a frequency of 0.5 Hz (1.95 ± 0.28 vs. 0.58 ± 0.05 ml/min; $P < 0.05$, repeated-measures ANOVA) and increased progressively at higher frequencies. FBF for the L-NAME group was significantly greater than the saline-infused animals at frequencies of 0.5 Hz and higher, resulting in a maximal 6.8-fold compared with a 3.6-fold increase over baseline at 2 Hz (Fig. 1*A*). Presumably, the higher systemic MAP contributed to this increase. Baseline hindlimb vascular resistance averaged 171.8 ± 15.4 $mmHg \cdot ml^{-1} \cdot min^{-1}$ in the absence of L-NAME and 221.7 ± 29.8 $mmHg \cdot ml^{-1} \cdot min^{-1}$ when L-NAME was

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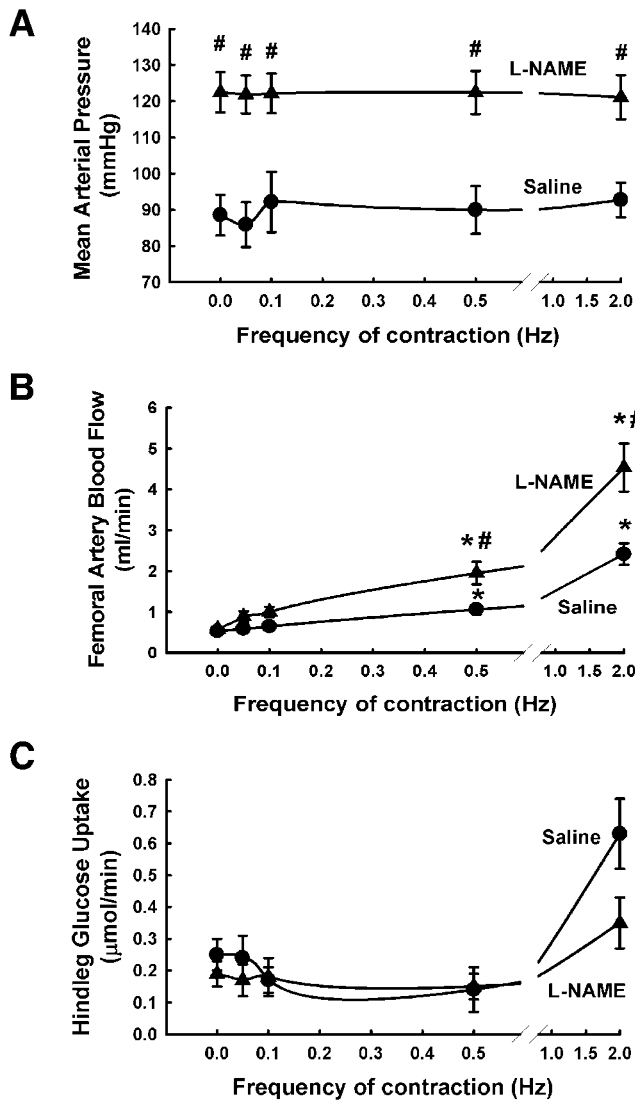

FIG. 1. Effect of L-NAME (▲; $n = 5$) or saline (●; $n = 6$) infusion on isometric contractile frequency–induced increases in MAP (*A*), total FBF (*B*), and hindlimb glucose uptake (*C*) (AV difference × flow). #$P <$ 0.05 vs. saline; *$P < 0.05$ vs. baseline (0 Hz).

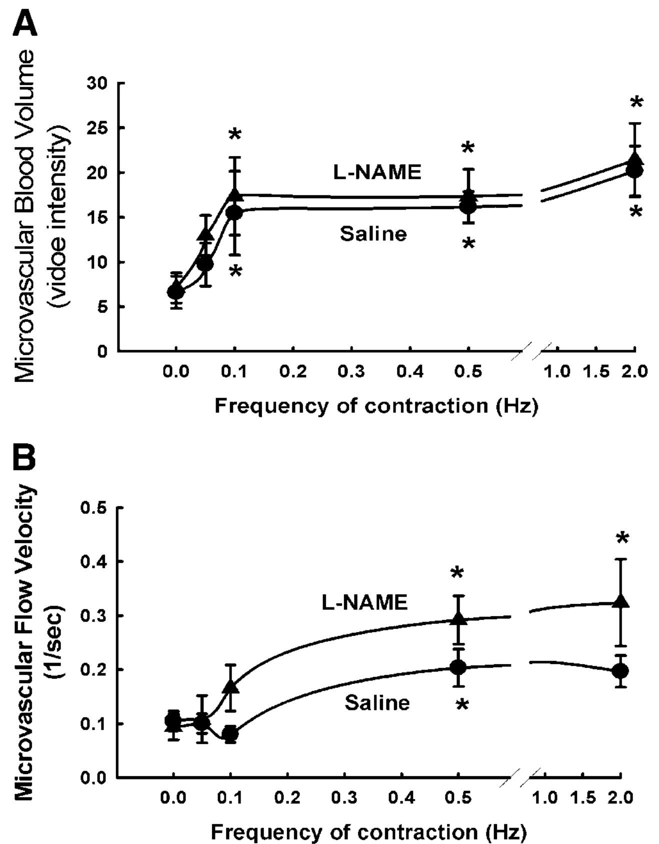

FIG. 2. Effect of L-NAME (▲; $n = 5$) or saline (●; $n = 6$) infusion on isometric contractile frequency–induced increases in MBV (*A*) and MFV (*B*). *$P < 0.05$ vs. baseline (0 Hz).

infused (not shown). In response to electrical stimulation at 0.1 Hz, vascular resistance declined in both groups, and resistance declined further in both groups as stimulation frequency increased.

The frequency-dependent response for MBV (measured by CEU) was markedly different from that of FBF (Fig. 2*A*). There was a greater than twofold increase in MBV in saline-infused animals by 0.1 Hz when compared with baseline (15.5 ± 4.7 vs. 6.6 ± 1.8 video intensity units; $P <$ 0.05). MBV only very modestly increased in response to further increases in frequency (~30% increase from 0.1 to 2 Hz). The frequency-dependent response of MBV for the L-NAME group was indistinguishable from that of the saline-infused group despite the differences in FBF at contraction frequencies >0.5 Hz. Microvascular flow velocity (MFV) as estimated from the CEU replenishment curve indicated that there was no increase at contraction frequencies up to 0.1 Hz, and in the saline-infused animals, there was even a small decline (Fig. 2*B*). At stimulation frequencies >0.5 Hz, MFV significantly increased in both saline- and L-NAME–infused animals compared with baseline ($P < 0.05$) without a significant difference between the two treatment groups. Inasmuch as MBF is approximated by the product of MFV and MBV, these findings are in accord with the increases in FBF found at higher frequency contractions. Hindlimb glucose uptake did not increase in these studies until higher frequency stimulation, and there were no significant differences in glucose uptake between the L-NAME and saline treatment groups (2 Hz) (Fig. 2*C*).

These observations suggest that microvascular recruitment within skeletal muscle is stimulated by contraction and that the frequency dependence for recruitment of new microvessels is different than that for increases in total femoral flow. This may indicate that different mechanisms are responsible for these two vascular responses. Inhibition of NOS with L-NAME was without effect on contraction-induced recruitment. Clearly, L-NAME exerted a vascular action based on the observed increases in MAP compared with saline-treated animals (122.4 ± 5.7 vs. 90 ± 6 mmHg; $P < 0.05$) and the higher FBF seen with L-NAME.

Protocol 2: low-frequency stimulation enhances EBD uptake by skeletal muscle. Based on the frequency response observed in protocol 1, we selected a low stimulation frequency (0.1 Hz) for subsequent studies of muscle EBD uptake. This low frequency robustly induced microvascular recruitment but did not increase FBF compared with baseline (0.59 ± 0.05 vs. 0.53 ± 0.03 ml/min; Fig. 1*A*). This was separately confirmed by stimulating the right hindlimb for 40 min at 0.1 Hz. Again, there was no increase in FBF over this longer stimulation duration (0.5 ± 0.1 ml/min at 40 min vs. 0.45 ± 0.05 ml/min at baseline; data not shown).

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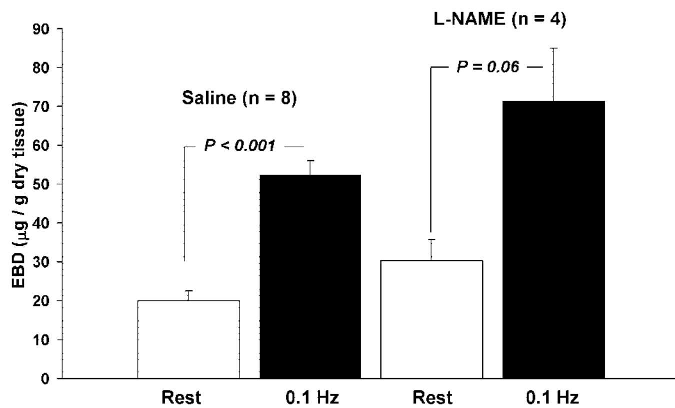

FIG. 3. Effect of L-NAME (n = 4) or saline (n = 8) infusion on 10 min of 0.1-Hz isometric frequency–induced increases in EBD extravasation in skeletal muscle.

In these studies, an intravenous bolus of EBD (25 mg/kg) circulated for 5 min before the onset of a 10-min bout of 0.1-Hz stimulation. Although separate muscle groups were isolated (soleus, tibialis, and gracilis) and analyzed, there were no significant differences in EBD content noted between fiber types, so data for EBD albumin were pooled from the 0.1 Hz–stimulated samples and compared with contralateral control. As shown in Fig. 3 for the saline group (n = 8), stimulation at a frequency of 0.1 Hz increased muscle EBD uptake 1.6-fold compared with the contralateral control leg (52.2 ± 3.8 vs. 20 ± 2.5 µg/g dry tissue; $P < 0.001$). Electrical stimulation likewise increased EBD content of the electrically stimulated compared with the contralateral hindleg in the L-NAME–treated group (n = 4). The muscle EBD content was modestly higher with L-NAME infusion in both the nonstimulated and stimulated legs when compared with the saline animals (P = 0.06). Again, as observed in protocol 1, the MAP was substantially elevated by the L-NAME infusion in animals studied under protocol 2 compared with saline-infused animals (139 ± 12 vs. 103.± 3 mmHg; $P <$ 0.05), indicating successful inhibition of NOS by L-NAME.

Because EBD itself fluoresces red when excited with a 568-nm frequency laser light, frozen sections of skeletal muscle from both 0.1 Hz–stimulated and nonstimulated hindlimbs were imaged to visualize the location of EBD within the tissue. This was done to ascertain whether the increased tissue EBD content measured in 0.1 Hz–stimulated muscle was due to increased amounts of EBD remaining in the lumen of recruited microvessels or represented an increase in EBD taken into tissue. The vascular endothelium was immunostained with a PECAM-1 antibody. As shown in Fig. 4, the PECAM-1 antibody clearly stains the innermost layer of the vessel wall corresponding to the vascular endothelium. There is little colocalization of EBD with PECAM-1. However, the red fluorescence intensity within muscle fibers was substantially greater in the stimulated versus the nonstimulated skeletal muscle samples. The dye was primarily concentrated in the elastin-rich perimysium around the vasculature in nonstimulated muscle, whereas dye reached further into fiber bundles in the 0.1 Hz–stimulated muscle (data not shown).

Protocol 3: low-frequency stimulation increases muscle ^{125}I-insulin content. Fig. 5 compares the skeletal muscle ^{125}I-insulin content 5 min after intravenous injection of 1.5 µCi ^{125}I-insulin in the electrically stimulated versus the lateral leg. Stimulation at 0.1 Hz significantly

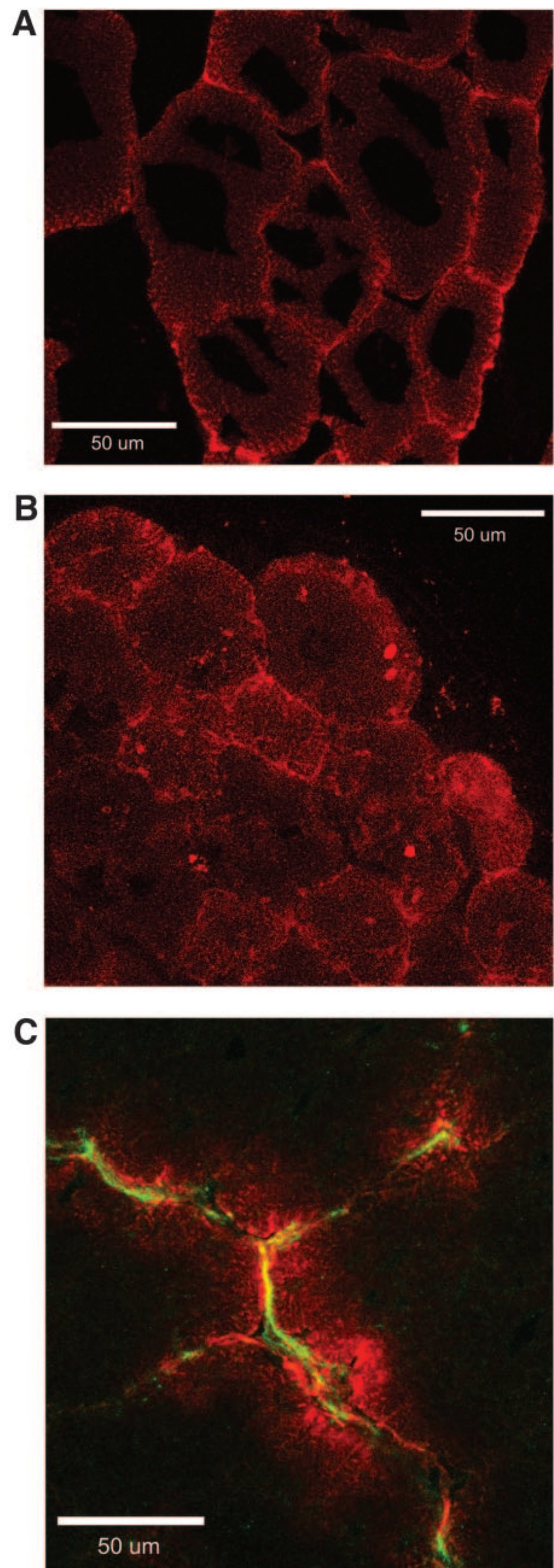

FIG. 4. Confocal images of EBD (red) content in control (nonstimulated) (A) and 0.1 Hz (10 min)–stimulated (B) skeletal muscle cryosections (tibialis). C: The endothelial cell is stained with an anti–PECAM-1 antibody (green) to highlight the vasculature, and EBD (red) traces albumin that leaves the vasculature and enters the surrounding muscle tissue.

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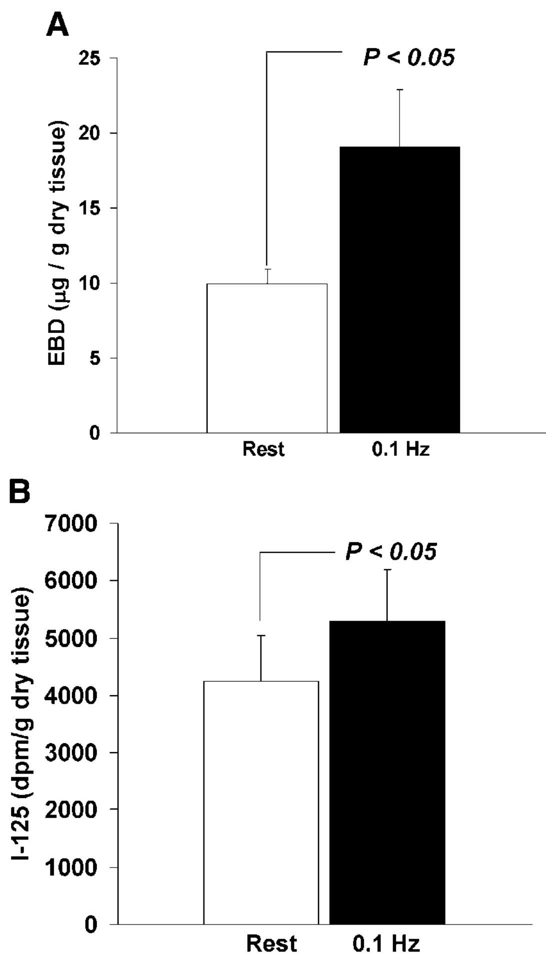

FIG. 5. Effect of 0.1-Hz stimulation (10 min) on muscle EBD (*A*) and ^{125}I-insulin (*B*) uptake (n = 6).

increased the tissue insulin content (5,299 ± 800 vs. 4,244 ± 903 dpm/g dry tissue; $P < 0.05$). In these animals, EBD was also infused beginning 15 min before harvesting tissue. We observed a 92% increase in EBD in the stimulated versus nonstimulated leg (19.1 ± 3.8 vs. 9.9 ± 1 μg/g dry tissue; $P < 0.05$). The shorter duration of insulin infusion was chosen because of the short circulating half-life (<5 min) for intact insulin.

DISCUSSION

In the present study, we observed that skeletal muscle contraction increased the skeletal muscle microvascular volume accessible to microbubbles at a lower frequency threshold (0.1 Hz) than that required to produce sustained increases in total FBF (>0.5 Hz). Our finding that there was a two- to threefold increase in MBV with 10 min of 0.1-Hz stimulation (Fig. 2*B*) underscores the sensitive nature of microvascular recruitment. Our present data agree with a previous report from our laboratory (34) that 2-Hz stimulation increased MBV ~twofold in addition to increasing FBF. The marked increases in MBV at stimulation frequencies much lower than those required to produce any sustained increases in total flow suggest that muscle contraction recruits microvessels via a mechanism that is distinct from that involving vasodilation of feed arterioles. We previously (29) reported that incremental increases in plasma insulin likewise recruit skeletal muscle capillaries at insulin concentrations lower than that required to increase total blood flow. However, although insulin-mediated increases in MBV are blocked and hindleg glucose uptake is partially blunted by infusion of the NO inhibitor L-NAME (33), in the present study, contraction-mediated increases are not. Although L-NAME at the dose used inhibits insulin-induced increases in MBV and in FBF, it had no effect on microvascular recruitment, and total flow was unchanged or increased in response to low-frequency contraction. We observed a similar rise in MAP with L-NAME when given with systemic insulin (33,39). Thus, L-NAME overcame the modest dilating action of insulin on resistance vessels. Compared with insulin, exercise is a much more potent stimulus to increase flow. It appears that the effect of exercise to locally vasodilate the exercising leg at a time when L-NAME has raised systemic pressure may account for the observed flow increase in the stimulated leg. We cannot exclude other possible systemic effects (e.g., sympatholysis) of L-NAME that may have contributed to vasodilation in the stimulated limb. Although differences among experimental preparation must be considered, this agrees with some (41,42) but not other (43,44) previous reports. These data suggest that low-frequency muscle contraction acts through an NO-independent mechanism distinct from that induced by insulin to recruit muscle microvasculature.

A greater endothelial surface area (i.e., increased MBV) is one of several variables that can promote movement of macromolecules across the capillary endothelium. This follows from consideration of Fick's Law, a simple model for diffusion of a solute across a membrane: $F = Q(A - I)(1 - e^{-PS/Q})$, where F is flux; Q is plasma flow; A and I are solute concentrations in the arterial and interstitial compartments, respectively; P is the permeability coefficient; and S is the available surface area (45,46). From this basic model, it is apparent that even in the absence of the changes of plasma (blood) flow, increases in either surface area or the permeability coefficient of the endothelial barrier will increase flux. We found that low-frequency contraction recruitment was accompanied by an increase of ~2.5-fold in EBD-albumin transfer from the vasculature of the stimulated compared with the nonstimulated contralateral hindlimb as measured by extravasated EBD content in skeletal muscle (Fig. 3). We have no independent measure of the "permeability coefficient" for albumin movement across the capillary endothelium. The majority of evidence suggests that this is a complex process involving a receptor-activated, caveolae-mediated transcytotic process (25,26,47) and may not be well described by the Fick formulation. However, the increases that we observed in EBD-albumin transport are consistent with what one would expect based on the increases in microvascular volume.

Immunostaining of frozen skeletal muscle sections showed that the systemic flush of ice-cold saline was effective in removing EBD from the intravascular compartment (Fig. 4). When compared with the nonstimulated control leg, it was clear that the 0.1 Hz–stimulated muscle fibers appeared to have a greater amount of EBD within muscle interstitium. We recognize the empiric nature of this flushing process. Although it is possible that both the endothelial cell and glycocalyx of the endothelial lumen could contribute to some of the dye content measured in muscle tissue, some of this may be lost in the flushing process. However, the confocal images verified that EBD that entered the interstitium of skeletal muscle and not dye left resident in the expanded microvasculature ac-

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counted for the increased EBD content. Our observation that even very low–frequency, short-duration isometric contraction markedly increases microvascular perfusion is consistent with the coupling between motor units and microvascular units proposed by Fuglevand and Segal (48).

Results obtained using ^{125}I-insulin as a tracer support the hypothesis that low-frequency contraction, in addition to recruiting capillaries and promoting albumin movement into muscle, also enhances insulin TET (Fig. 5). Recent results from our laboratory indicate that as with albumin, the movement of insulin across the endothelium is a receptor-mediated process that may involve caveolae (49). The increase in TET of insulin is less dramatic than that for albumin. It is likely that this is at least in part due to the shorter time interval (5 min) selected for the insulin compared with the 15 min used with EBD. The 5-min infusion time was selected to minimize the time available for metabolism of the iodinated insulin. We confirmed that at 5 min after administration of the ^{125}I-insulin, 90% of the radioactivity in plasma remained bound to the insulin (as estimated by trichloroacetic acid precipitation). These data suggest that low-frequency contraction acutely enhanced the transfer of intact iodinated insulin across the capillary endothelium, as was the case observed for the EBD-albumin complex. However, our results do not discount the possibility that low-frequency contraction may also induce changes in the luminal composition of the capillary endothelium (e.g., altered glycocalyx profile, increased presence of insulin receptors at the membrane, increased numbers of caveolae, etc.), which could also explain the observed increase in ^{125}I-insulin content in muscle tissue.

In conclusion, our data demonstrate that brief (0.5-ms duration) low-frequency (0.1 Hz) muscle contraction fully recruits the microvasculature via an NO-independent pathway in the absence of any measurable increase in total blood flow. Furthermore, contraction-mediated recruitment promotes the egress of both albumin and ^{125}I-insulin from the microvasculature to the muscle. L-NAME did not inhibit muscle uptake of EBD-bound albumin stimulated by low-frequency contracted muscle versus control and even revealed a modest trend upward. However, the duration of microvascular recruitment persistence after acute bouts of exercise or contraction is currently unknown, and it remains to be determined whether NOS inhibition will have an effect on ^{125}I-insulin content in 0.1 Hz–stimulated versus control skeletal muscle. Although our results suggest that this enhanced transport of both albumin and ^{125}I-insulin is associated with increased capillary surface area (MBV), we cannot exclude the possibility that low-frequency contraction might also induce other TET pathways. However, it is clear that low-frequency muscle contraction can facilitate nutrient and hormone delivery to skeletal muscle by providing a greater nutritive flow in vivo. The observation that contraction-enhanced insulin sensitivity is associated with improved microcirculation (19) is of potential importance for our understanding of the beneficial effects of acute exercise, and this potential is underscored by recent human studies demonstrating dramatic microvascular recruitment by low-frequency, low-intensity exercise (50). These findings may, in part, explain the enhanced insulin-mediated glucose disposal that accompanies exercise.

ACKNOWLEDGMENTS

A.C.I. has received a predoctoral fellowship grant (0615460U) from the American Heart Association. E.J.B. has received National Institutes of Health Grants DK-57878 and DK-063609.

The University of Virginia Histology Core assisted in the preparation of frozen sections of muscle tissue. We thank Dr. Brian Duling and Katherine Day for help with the confocal imaging.

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