Exercise adaptation attenuates VEGF gene expression in human skeletal muscle


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Angiogenesis is a component of the multifactorial adaptation to exercise training, and vascular endothelial growth factor (VEGF) is involved in extracellular matrix changes and endothelial cell proliferation. However, there is limited evidence supporting the role of VEGF in the exercise training response. Thus we studied mRNA levels of VEGF, using quantitative Northern analysis, in untrained and trained human skeletal muscle at rest and after a single bout of exercise. Single leg knee-extension provided the acute exercise stimulus and the training modality. Four biopsies were collected from the vastus lateralis muscle at rest in the untrained and trained conditions before and after exercise. Training resulted in a 35% increase in muscle oxygen consumption (V˙O₂ max) (16, 27, 30). Although vascular endothelial growth factor (VEGF) is likely involved in the regulation of this angiogenic process (19, 22), the molecular basis before the study and, as indicated by the mean V˙O₂ max measured during conventional cycle ergometer exercise (V˙O₂ max = 33.9 ± 1.8 ml·kg⁻¹·min⁻¹), they were appropriately classified as sedentary. It is interesting to note that subject AG was somewhat of an outlier (obese, elevated untrained citrate synthase values, and high blood pressure).

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EXERCISE TRAINING LEADS to many skeletal muscle adaptations, including an increased capillary network (8, 13, 37). As O₂ transport conductance is positively related to the number of capillaries per muscle fiber, it is likely that angiogenesis plays an important role in the facilitation of O₂ transport to skeletal muscle mitochondria and, therefore, may influence maximal oxygen consumption (V˙O₂ max) (16, 27, 30). Although vascular endothelial growth factor (VEGF) is likely involved in the regulation of this angiogenic process (22, 36), the molecular basis for this important exercise adaptation is far from being well understood. In fact, how VEGF mRNA levels vary in response to an exercise stimulus in an untrained vs. an exercise-trained state, in which many of the metabolic/morphometric adaptations occur, has not even been addressed.

Two recent investigations documented that VEGF is upregulated in human skeletal muscle in response to a single bout of dynamic exercise (12, 31). Studying intact rats, Breen et al. (7) documented an elevation in skeletal muscle VEGF mRNA after a single exercise bout, while Hang et al. (13) supported this finding in electrically stimulated rat skeletal muscle. Similar but lesser effects of exercise were reported for basic fibroblast growth factor (bFGF) (7), which is also recognized as a direct angiogenic factor (11). However, because VEGF contains a signal sequence peptide and can be secreted from cells, it is more likely to be directly related to angiogenic control (19, 22).

If VEGF is directly involved in the angiogenic response to exercise, it seems likely that a negative feedback mechanism would exist to reduce the level of VEGF gene expression as exercise adaptation occurs. Thus, to further determine the role of VEGF in the response to exercise, this study proposes to 1) document the adaptations associated with exercise training of sedentary human skeletal muscle and 2) test the hypothesis that, in the face of such adaptations, the VEGF mRNA increase in response to acute exercise will be significantly attenuated.

METHODS

Subjects. The untrained VEGF mRNA response to exercise data collected from these subjects was recently published (31) and act as reference data in this paper. Six sedentary males [weight 79.8 ± 2.9 kg, age 26.7 ± 1 yr, and height 175.3 ± 3.2 cm (mean ± SD)] volunteered to participate in the present study after health histories and physical examinations were completed and after informed, written consent was obtained in accordance with the University of California, San Diego, Human Subjects Committee requirements. None of the subjects had performed endurance exercise on a regular basis before the study and, as indicated by the mean V˙O₂ max measured during conventional cycle ergometer exercise (V˙O₂ max = 33.9 ± 1.8 ml·kg⁻¹·min⁻¹), they were appropriately classified as sedentary. It is interesting to note that subject AG was somewhat of an outlier (obese, elevated untrained citrate synthase values, and high blood pressure).
in the otherwise homogeneous group. These differences are consistent with the VEGF mRNA results obtained in subject AG.

**Experimental design.** After recruitment and familiarization with the equipment, subjects performed one exercise bout on the single leg knee-extensor ergometer. Both the resting (right) and exercised (left) legs were biopsied 1 h after the exercise stimulus. Skeletal muscle VO$_2$ max during knee-extensor exercise was then measured in catheter-based studies. Eight weeks of single leg knee-extensor training was then performed. At the completion of this training period, the catheter studies were repeated, and the left leg (trained) was biopsied in the resting state and after an exercise bout.

**Exercise apparatus.** The knee-extensor ergometer used to produce an acute exercise stimulus was designed to limit exercise to the quadriceps muscles of the left leg (27). Briefly, subjects were semirecumbent in an adjustable chair, with a special ankle boot on their left leg, which was connected to the ergometer by a bar (see Fig. 1 in Ref. 28). Contractions of the quadriceps muscles caused the lower part of the leg to extend from 90° to 170°. Therefore, the lower leg traveled in an arc-shaped trajectory of ~80°. The momentum of the ergometer passively returned the relaxed leg to the start position, and, as a result, the quadriceps muscle was functionally isolated (26). During exercise, the contraction rate was maintained at 60 contractions/min.

**Exercise stimulus.** One week after a brief familiarization period, subjects underwent an acute knee-extensor exercise bout using their left leg. This consisted of a 5-min, unloaded warmup, followed by a graded maximal exercise test with a 10-min rest, which was then followed by 30 min of knee-extensor exercise at 50% of the maximum work rate achieved. The graded exercise test required subjects to maintain each work rate for 60 s, after which the work rate was increased in increments of 5 W. Subjects continued the test until they were unable to maintain a cadence of 60 repetitions/min for the entire run. This complete procedure was reproduced at the end of the 8-wk training regimen.

**Exercise training.** The knee-extensor ergometer was used for exercise training of the quadriceps muscles of the left leg (27). Training was performed three times per week for 8 wk, with each exercise session lasting 1 h. The training regimen varied within repeating 2-wk cycles that included high-intensity interval sessions, long slow distance, fartlek, and time trial-type workouts. Hence, the exercise program was designed to provide a variety of stimuli to promote optimum improvement in exercise capacity. The last session of each 2-wk period included a graded exercise test that reassessed the maximum work rate of each subject. Work rates for the next 2-wk period were increased according to the results of this test to ensure a continued training stimulus.

**Muscle biopsies.** Approximately 50–70 min after completion of the pretraining exercise bout, a muscle biopsy was taken from both the exercised (left) and rested (right) legs. Eight weeks later, a muscle biopsy was again taken from the now-trained left leg 50–70 min after exercise and then again from the same leg after a 48-h rest period. As peak work rate increased due to training, the posttraining exercise bout was performed at the same relative work rate as before training (50% of work rate maximum). This resulted in a greater posttraining absolute work rate. All biopsies were taken from the vastus lateralis, ~3.5-cm deep, 15-cm proximal to the knee, and slightly distal to the ventral midline of the muscle. The 5-mm diameter biopsy needle was attached to sterile tubing and a syringe that applied a negative pressure to assist in the muscle sample collection (14). Lidocaine (2%) was used as local anesthetic and was infiltrated beyond the depth of the biopsy. Muscle samples from each biopsy were either immediately frozen in liquid nitrogen and stored at −80°C for citrate synthase assay or prepared for morphometric analysis. In summary, muscle biopsies were collected from the vastus lateralis in four different states: 1) untrained-rested, 2) untrained-acutely exercised, 3) trained-rested, and 4) trained-acutely exercised.

**RNA isolation and Northern analysis.** Total cellular RNA was isolated from each muscle sample (muscle sample mass range: 65–110 mg) using the method of Chomczynski and Sacchi (9). RNA preparations were quantitated by absorbance at 260 nm, and intactness was assessed by ethidium bromide-staining, after separation by electrophoresis in 6.6% formaldehyde-1% agarose gel. Fractionated RNA was transferred by Northern blot to Zeta probe membrane (Bio-Rad Laboratories, Hercules, CA). RNA was cross-linked to the membrane by ultraviolet irradiation for 1 min and stored at 4°C. The blots were then probed with oligolabeled [α-32P]deoxyctydidylic triphosphate dCDNA probes, which had a specific activity ≥1 × 106 disintegrations min$^{-1}$ μg DNA$^{-1}$ (10). Human VEGF is a 0.93-kb cDNA fragment isolated from the EcoRI site of pUC-derived plasmid (22). The bFGF is a 1-kb Xho I fragment of human bFGF cDNA (21). Prehybridization and hybridization were performed in 50% formamide, 5 × SSC (20 × SSC is 0.3 M sodium chloride, 0.3 M sodium citrate), 10 × Denhardt’s solution (100 × Denhardt’s solution is 2% Ficoll, 2% polyvinylpyrrolidone), 50 mM sodium phosphate (pH 6.5), 1% sodium dodecyl sulfate, and 250 μg/ml salmon sperm DNA at 37 or 42°C. Blots were washed with 2 × SSC and 0.1% sodium dodecyl sulfate at 50°C for the VEGF mRNA. Blots were exposed to XAR-5 X-ray film (Eastman Kodak, New Haven, CT) by use of a Cronex Lightning Plus screen at −70°C. Autoradiograms were quantitated by densitometry (Media Cybernetics, Silver Spring, MD). Each blot was subsequently reprobed (after prior complexes were stripped) with a cDNA specific for 18S ribosomal RNA, and this signal was used to normalize the mRNA signal for minor variations in lane loading. All samples from a single subject were run on the same gel, producing optimum conditions for quantitative analyses across conditions.

**Skeletal muscle VO$_2$.** Subjects reported to the laboratory before and after the 8-wk training regimen to perform the catheter studies. Each time, two catheters (radial artery and left femoral vein) and a thermocouple (left femoral vein) were placed using sterile technique, as previously reported (25, 29). To measure blood flow during exercise, iced saline was infused intermittently through the femoral venous catheter at flow rates sufficient to decrease femoral venous blood temperature at the thermocouple by −1°C. Infusions were continued for 15–20 s until femoral vein temperature had stabilized at its new lower value. Saline injection rate was measured by weight change in a reservoir bag suspended from a force transducer that was calibrated before and after each experiment. The calculation of blood flow was performed on thermal balance principles detailed by Andersen and Saltin (4). Before each blood flow measurement, 3- to 4-ml samples of arterial and femoral venous blood were withdrawn from the catheters anaerobically to measure PO$_2$, PCO$_2$, pH, O$_2$ saturation, and hemoglobin concentration ([Hb]). All measurements were made on an IL 1306 blood gas analyzer and IL 482 co-oximeter (Instrumentation Laboratories, Lexington, MA.) Between each sample, electrodes were calibrated and demonstrated acceptable reproducibility (SD of repeated determinations: PO$_2$ and Pco$_2$, 1.5 mmHg; pH, 0.003). O$_2$ concentration ([O$_2$]) was calculated as 1.39 ml O$_2$ × [Hb]/100 ml × measured O$_2$ saturation (%). 0.003 ml O$_2$/100 ml of blood × measured PO$_2$ (mmHg). Arterial-venous
[O$_2$] difference was calculated from the difference in radial artery and femoral venous [O$_2$]. This difference was then divided by arterial concentration to give O$_2$ extraction. Muscle VO$_2$ was calculated as the product of blood flow and arterial-venous [O$_2$] difference, whereas O$_2$ delivery was calculated as the product of blood flow and arterial [O$_2$].

In these studies, the work rate was increased from an unweighted warmup to 25, 50, 75, 90, and 100% of the previously determined maximum work rate, followed by progressive 5% increases in work rate to ensure that a true maximum was achieved. Data were obtained at each level, which was maintained for 2–3 min, but only maximum values are reported herein. Each exercise bout was completed in 9–15 min.

Citrate synthase activity (CSA). From a portion of each muscle biopsy the CSA was determined spectrophotometrically at 30°C, in a Milton Roy model 21D spectrophotometer by the method of Srere (34). Briefly, the tissue was homogenized in an ice bath using a glass Konte Dural tissue grinder mounted on a variable-speed drill. The homogenate was diluted with EDTA and EGTA phosphate buffer to a convenient concentration, and spectrophotometry was performed in a Tris buffer. The acetyl-CoA reagent was in the form of a sodium salt. The homogenate from each sample was divided into three duplicate samples and assayed in triplicate. The coefficient of variation of CSA across triplicates of the same sample was 0.06%. Within each subject, all samples were analyzed in a random order, and all samples from a given subject were analyzed at the same time.

Morphometric analyses. Muscle biopsy samples were oriented to facilitate transverse sectioning, mounted on an embedding medium, and then frozen in liquid nitrogen. Serial transverse sections (8-μm thick) were cut in a cryostat at −25°C. Sections were stained to identify capillaries using the amylase periodic acid Schiff technique first reported by Andersen in 1975 (1). Briefly, sections were fixed in Carnoy’s fixative (10 min, 21°C) and rinsed in distilled water. The glycogen was then digested with 1% amylase and again rinsed in distilled water. The sections were then oxidized in 1% periodic acid (10 min, 21°C), stained with Schiff’s reagent, rinsed in water, and dehydrated. Fiber area and the number of capillaries around a fiber were measured using an image analysis system (V150, American Innovations). An average of 56 ± 13 fibers were sampled from each section.

Muscle O$_2$ transport conductance and mean capillary Po$_2$ calculations. Maximal muscle O$_2$ transport conductance (DO$_2$) and mean capillary Po$_2$ at maximal exercise were calculated as described previously (35). Briefly, a numerical integration procedure is used to determine the value of DO$_2$, assumed constant along the capillary, that produces the measured femoral venous Po$_2$, given the measured arterial Po$_2$ and an intracellular Po$_2$ assumed close to zero. An additional, and at this time unavoidable, assumption of this calculation is that the only explanation of O$_2$ remaining in the femoral venous blood is diffusion limitation of O$_2$ efflux from the muscle microcirculation. Perfusion/VO$_2$, heterogeneity and perfusional or diffusional shunt are considered negligible. To the extent that these phenomena do not contribute O$_2$ to femoral venous blood, the parameter DO$_2$ is a conductance coefficient that expresses the diffusing capacity required to achieve the measured VO$_2$ max, assuming only diffusion limitation. This assumption cannot be currently avoided due to the lack of specific means for detecting Perfusion/VO$_2$ heterogeneity and shunt. Mean capillary Po$_2$ is the numerical average of all Po$_2$ values computed, equally spaced in time, along the capillary from the arterial to the venous end.

**Statistical analysis.** Rested and acutely exercised muscle in both the untrained and trained states were quantitatively assessed using densitometry values of VEGF and bFGF mRNA northern blots (normalized by the 18S band) using a repeated measures ANOVA. Differences between groups were then identified using a Newman-Keuls post hoc analysis. Functional and morphometric measurements in the untrained and trained state were compared using paired t-tests. Statistical significance was accepted if $P < 0.05$. All data are presented as means ± SE.

**RESULTS**

Functional and morphometric response to chronic knee-extensor training. The average maximal knee-extensor work rate increased 33% from the untrained to the trained state (Table 1). This was accompanied by a 34% increase in skeletal muscle VO$_2$ max after training (Table 1). This increase in VO$_2$ max was achieved by a small (9%) but significant increase in muscle blood flow and a large increase in maximal O$_2$ extraction (21%; Table 1). Maximal O$_2$ conductance also increased significantly (34%), whereas calculated mean capillary Po$_2$ remained unchanged. CSA, an indicator of mitochondrial capacity, increased 18.2% from the untrained to trained state (Table 1). Structural changes in the muscle sampled before and after the training period were consistent with knee-extensor training demanding a strength component, resulting in an increase in fiber area (18%). There was also a significant angiogenic response, with an 18% increase in the number of capillaries around each muscle fiber. However, because both fiber area and the number of capillaries/fiber increased similarly, capillary density was unaltered. In combination, these data clearly illustrate that the 8-wk training regimen resulted in significant improvements in O$_2$ transport through increased capillarity, O$_2$ conductance, O$_2$ extraction, and muscle blood

### Table 1. Functional, morphometric, and growth factor data for the quadriceps muscles in untrained and exercise-trained states

<table>
<thead>
<tr>
<th></th>
<th>Untrained</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal work rate, W</td>
<td>64 ± 5</td>
<td>85 ± 6*</td>
</tr>
<tr>
<td>Muscle VO$_2$ l/min</td>
<td>0.52 ± 0.05</td>
<td>0.70 ± 0.06*</td>
</tr>
<tr>
<td>Maximal muscle blood flow, l/min</td>
<td>4.36 ± 0.52</td>
<td>4.74 ± 0.56*</td>
</tr>
<tr>
<td>Maximal O$_2$ delivery, l/min</td>
<td>0.91 ± 0.12</td>
<td>0.98 ± 0.12*</td>
</tr>
<tr>
<td>Maximal O$_2$ extraction, %</td>
<td>60.1 ± 4.7</td>
<td>72.8 ± 3.5*</td>
</tr>
<tr>
<td>Maximal O$_2$ conductance, ml min$^{-1}$ mmHg$^{-1}$</td>
<td>16.5 ± 1.7</td>
<td>20.0 ± 1.4*</td>
</tr>
<tr>
<td>Mean capillary Po$_2$, mmHg</td>
<td>41.4 ± 1.5</td>
<td>39.6 ± 1.1</td>
</tr>
<tr>
<td>CSA, μmol min$^{-1}$ g tissue$^{-1}$</td>
<td>12.3 ± 0.3</td>
<td>14.5 ± 0.3*</td>
</tr>
<tr>
<td>Number of capillaries/fiber</td>
<td>3.63 ± 0.17</td>
<td>4.29 ± 0.55*</td>
</tr>
<tr>
<td>Fiber area, μm$^2$</td>
<td>4,683 ± 659</td>
<td>5,536 ± 580*</td>
</tr>
<tr>
<td>VEGF/18S rested</td>
<td>0.4 ± 0.04</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>VEGF/18S postexercise</td>
<td>16.9 ± 6.7</td>
<td>5.9 ± 1.4*</td>
</tr>
<tr>
<td>bFGF/18S rested</td>
<td>0.9 ± 0.07</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>bFGF/18S postexercise</td>
<td>0.8 ± 0.07</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>

Functional data are at maximal exercise. All data are means ± SE. VEGF/18S and bFGF/18S values are presented in arbitrary units. V0$_2$ max, maximal oxygen consumption; CSA, citrate synthase activity; VEGF, vascular endothelial growth factor; 18S, 18S ribosomal RNA; bFGF, basic fibroblast growth factor. *Significantly different from the untrained state ($P < 0.05$).
flow, all of which translate to a large increase in skeletal muscle \( \dot{V}O_2 \text{max} \).

**VEGF response to acute exercise in untrained and trained muscle.** As illustrated in Figs. 1 and 2, it is clear that the VEGF mRNA levels of rested human muscle is relatively low. Furthermore, the level of VEGF mRNA in rested muscle is not altered by the transition from the untrained to trained state (Figs. 1 and 2). It is also evident that VEGF mRNA abundance in human skeletal muscle increased significantly 1 h after an acute small muscle mass exercise bout (Table 1, Figs. 1 and 2). However, after an 8-wk exercise training program (specific to this muscle group), performance of the same relative, but significantly increased, absolute work rate for 30 min resulted in a VEGF mRNA level that was significantly elevated above resting levels. However, this response was significantly attenuated compared with the pretraining response (Table 1, Figs. 1 and 2).

**bFGF response to acute exercise in untrained and trained muscle.** Resting levels of bFGF mRNA in both the untrained and trained conditions (0.9 ± 0.07 vs. 1.3 bFGF/18S arbitrary units, respectively) were unaffected by exercise in both the untrained and trained states (0.8 ± 0.7 and 1.1 ± 0.2 bFGF/18S arbitrary units, respectively; Table 1). Thus, in contrast to the VEGF mRNA response, bFGF mRNA response to exercise in both the untrained and trained states was not measurably different from control values (Table 1).

**DISCUSSION**

The principal finding of this study is that the significant and substantial increase in VEGF mRNA in untrained human skeletal muscle after a single bout of exercise is attenuated in trained human skeletal muscle (Table 1, Figs. 1 and 2). This attenuation in the VEGF response was observed despite the fact that the same relative exercise stimulus was performed at a 30% greater absolute work rate due to a training-induced increase in maximum work capacity (Table 1). This observation supports the concept that increased VEGF mRNA levels are necessary in the untrained state to promote angiogenesis and thus to facilitate the observed increase in \( O_2 \) extraction, \( O_2 \) conductance, blood flow, and \( \dot{V}O_2 \text{max} \). However, in the trained state, the achievement of this capillary proliferation and its associated benefits leads to an attenuation of this process, suggesting a negative feedback mechanism (Table 1, Fig. 3).

**VEGF response to exercise.** A significant increase in VEGF mRNA abundance in human skeletal muscle in response to an acute exercise stimulus has previously been demonstrated (12, 31). Because VEGF functions as a direct angiogenic factor with a high specificity for vascular endothelial cells (22), these findings are in line with the theory that VEGF is involved in the formation of new blood vessels within human skeletal muscles in response to exercise (8, 13, 37). This is an essential adaptive response in skeletal muscle to repeated exercise (i.e., training) that results in an increase in the number of capillaries per muscle fiber, which enhances \( O_2 \) transport conductance between the microcirculation and mitochondria (5). The present data extend the link between VEGF and capillary proliferation to include the observation that after significant adaptations to exercise training, including angiogenesis, the previously large VEGF mRNA response to acute exercise is significantly attenuated (Table 1, Figs. 1 and 2). This finding adds credence to the con-
cept that VEGF is important in the initial phase of exercise adaptation, but, when significant angiogenesis has occurred due to training, the need and importance of this mechanism becomes significantly reduced. Unfortunately, because of the limited number of sampling times used, the present study cannot accurately determine the quantity or duration of training at which this attenuated VEGF mRNA response occurs. It is possible that this reduced VEGF response occurs much earlier than after 8-wk of exercise training. The answer to this question awaits further studies, perhaps using an animal model in which the determination of a time course is more feasible than in human studies.

bFGF response to exercise. Exercise in both the untrained and trained states did not lead to an increased bFGF mRNA level. Thus, in agreement with the findings and inferences of other work, these data support the concept that bFGF may play a less significant role in the control of the angiogenic process in skeletal muscle than VEGF (7, 13, 19, 22). However, it should be recognized that this study is limited by the single muscle sample taken 1 h after exercise and bFGF may increase beyond the measured time frame, although this was not evident in a previous exercise study in rats that used several sampling times after exercise (7).

Physiological adaptations to single leg knee-extensor exercise. Much is known about the physiological adaptations to whole body endurance exercise in which \( \dot{V}O_2 \text{max} \) is elevated by increases in both cardiac output and arteriovenous \([O_2]\) content difference (6). Little is known about the extent of the physiological adaptations to endurance exercise that occur in a relatively small muscle mass exercise that minimally taxes central factors (2). Previously, endurance training of a reduced muscle mass has included the whole leg and thus resulted in some improvements in the central circulation (32). Although the quadriceps muscles are often the focus of strength training studies (18), data on endurance training of only those muscles is sparse. In the only study found, Magnusson et al. (23) trained patients with chronic heart failure for 8 wk and reported a high degree of adaptation to the small muscle mass training that lead to a 43% increase in peak workload and large increases in enzymatic markers of mitochondrial content. Unfortunately, no control subjects were assessed in that study, but, in combination with the present study, it appears that relatively large increases in \( \dot{V}O_2 \text{max} \) and maximal work rate can be achieved by endurance training a functionally isolated muscle in both health and disease. The increase in \( \dot{V}O_2 \text{max} \) (35%) reported here is certainly at the upper

Fig. 3. Evidence that exercise training increases the number of capillaries/fiber and is associated with increased quadriceps \( \dot{V}O_{2\text{max}} \), maximal \( O_2 \) extraction, and maximal \( O_2 \) conductance, whereas VEGF gene expression in response to acute exercise is attenuated by training. The attenuated VEGF gene response, in the face of significant structural and functional adaptations to training, suggests the existence of a negative feedback mechanism that limits capillary growth as structural changes enhance function and supports the importance of the initial VEGF response in achieving these adaptations. * Significantly different from the untrained state \((P < 0.05)\).
end of the often-reported 6–20% range, seen with whole body exercise training (24). Certainly, this form of exercise training resulted in significant peripheral adaptations such as capillary proliferation (3) and increased CSA (33), paralleling the findings of previous whole body training studies, but, in this scenario, they appear to constitute the bulk of the adaptation, as muscle blood flow was only minimally increased.

The positive effects of exercise training on \( O_2 \) transport and utilization and capillarity in skeletal muscle contrast quite starkly with the attenuated VEGF response to a single bout of exercise (Table 1, Fig. 3). These data are suggestive of the fact that VEGF plays a role in determining the structural capacity for \( O_2 \) flux but that this role is reduced as the increased capacity for \( O_2 \) flux is achieved. However, it is interesting to note that these data do not support the classical view that diffusion distance plays an important role in determining \( O_2 \) flux (20), as there was an equal increase in muscle fiber area and the number of capillaries/fiber, whereas \( O_2 \) conductance was markedly increased. This is consistent with the observation that a substantial gradient exists between blood and muscle cell (28), suggesting that the major resistance to \( O_2 \) flux from red cell to mitochondria is in this short diffusion distance. The limited importance of diffusion distance was recently highlighted by work from our laboratory that revealed similar \( O_2 \) conductance in normal and immobilized dog muscle, despite a 45% decrease in fiber area in the latter condition (15). Thus mounting evidence, including the present data, supports the concept that the capillary-to-fiber interface, and not the diffusion distance, appears to be most critical to \( O_2 \) flux capacity (17). This understanding supports the increase in \( O_2 \) conductance and \( O_2 \) extraction caused by the elevated number of capillaries/fiber that accompanied training and facilitated the increased \( V_O_2_{max} \). In addition, these findings highlight the initial demand for VEGF-mediated angiogenesis in the untrained state and the attenuation of the VEGF response when structure and function adequately responded to the repeated exercise stimulus (Fig. 3).

**Limitations to the present study.** Varied growth factor response times between, and even within, growth factors (perhaps due to training) and possible regional differences within the muscle may lead to an erroneous conclusion based on one sample. In the future, multiple biopsy samples, which will reveal both time course issues and information beyond transcription to translation of angiogenic growth factors and receptors, need to be taken. Additionally, multiple muscle sample times throughout the 8-wk training period would have allowed a more accurate determination of the time at which the VEGF upregulation is attenuated due to the regular exercise stimuli and the degree of angiogenic response. Additionally, because of a limited total number of biopsies, the rested sample in the untrained state was taken from the right leg, whereas the rested sample in the trained state was taken from the left leg. This decision was supported by the concept that these sedentary individuals should have little difference in everyday leg usage. Although these two sets of samples were not statistically different from each other, the variance was much greater in the exercise-trained, but rested, leg. Thus, as we are unsure of the difference between VEGF mRNA levels between the right and left legs, this difference cannot be positively ascribed to the training. Such limitations, as in this study, are ethically unavoidable due compliance issues with human subjects.

In summary, we documented that the large increase in VEGF mRNA in untrained human skeletal muscle is attenuated in exercise-trained muscle. In contrast, this pattern of mRNA increase was not mirrored by bFGF, which did not vary with acute exercise in the either the untrained or trained state. The increased \( O_2 \) extraction, \( O_2 \) conductance, blood flow and muscle \( V_O_2_{max} \), accompanied by morphometric evidence of capillary proliferation due to training, support the concept that the increased abundance of VEGF mRNA after acute exercise in the untrained state plays a role in instigating the angiogenic response to exercise. This link between VEGF and structural and functional adaptation to exercise training was further supported by the finding that VEGF mRNA upregulation in exercise-adapted muscle, with an already elevated number of capillaries, appears to be less necessary and is therefore significantly attenuated in the trained state.

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