Myocyte vascular endothelial growth factor is required for exercise-induced skeletal muscle angiogenesis

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Submitted 27 May 2010; accepted in final form 30 July 2010

Olfert IM, Howlett RA, Wagner PD, Breen EC. Myocyte vascular endothelial growth factor is required for exercise-induced skeletal muscle angiogenesis. Am J Physiol Regul Integr Comp Physiol 299: R1059–R1067, 2010. First published August 4, 2010; doi:10.1152/ajpregu.00347.2010.—We have previously shown, using a Cre-LoxP strategy, that vascular endothelial growth factor (VEGF) is required for the development and maintenance of skeletal muscle capillarity in sedentary adult mice. To determine whether VEGF expression is required for skeletal muscle capillary adaptation to exercise training, gastrocnemius muscle capillarity was measured in myocyte-specific VEGF gene-deleted (mVEGF−/−) and wild-type (WT) littermate mice following 6 wk of treadmill running (1 h/day, 5 days/wk) at the same running speed. The effect of training on metabolic enzyme activity levels and whole body running performance was also evaluated in mVEGF−/− and WT mice. Posttraining capillary density was significantly increased by 59% (P < 0.05) in the deep muscle region of the gastrocnemius in WT mice but did not change in mVEGF−/− mice. Maximal running speed and time to exhaustion during submaximal running increased by 20 and 13% (P < 0.05), respectively, in WT mice after training but were unchanged in mVEGF−/− mice. Training led to increases in skeletal muscle citrate synthase (CS) and phosphofructokinase (PFK) activities in both WT and mVEGF−/− mice (P < 0.05), whereas β-hydroxyacyl-CoA dehydrogenase (β-HAD) activity was increased only in WT mice. These data demonstrate that skeletal muscle capillary adaptation to physical training does not occur in the absence of myocyte-expressed VEGF. However, skeletal muscle metabolic adaptation to exercise training takes place independent of myocyte VEGF expression.

OBSERVATIONAL STUDIES SUPPORT vascular endothelial growth factor (VEGF) expression as an important factor for regulating skeletal muscle angiogenesis in both humans and animals (7, 19–21, 47, 49, 50, 52). In patients with chronic disease conditions (e.g., chronic obstructive pulmonary disease, heart failure, and diabetes), as well as aging, locomotor skeletal muscle VEGF expression has also been reported to be lower (3, 33, 34, 51), and these individuals often exhibit muscle weakness, reduced physical activity, and loss of skeletal muscle vascular density (1, 10, 24, 41, 45, 61). A similar phenotype is found in sedentary (untrained) myocyte-specific VEGF gene-ablated mice, which exhibit impaired exercise capacity and >50% loss of skeletal muscle microvessel density (48, 56). These data demonstrate the importance of the myocyte as a source of VEGF in determining basal skeletal muscle capillarity and also imply that paracrine secretion of VEGF from other organs or cells is not sufficient to preserve skeletal muscle microvessel density in the absence of VEGF from the myocyte. However, it remains unknown whether the mechanisms regulating capillary maintenance and exercise-induced angiogenesis involve the same factors produced by skeletal myocytes. It is possible that the mechanism for maintaining capillaries (and/or preventing capillary regression of already established capillaries) is not the same mechanism as that eliciting the formation of new capillaries in response to exercise-related stimuli. Although there is an abundance of evidence demonstrating that VEGF is responsive to exercise, no study to date has been able to directly determine whether myocyte VEGF is specifically required for the well-known skeletal muscle angiogenic response to exercise training. For example, can exercise training induce other angiogenic mitogens to compensate in absence of VEGF, or could VEGF expressed by other cell types compensate for the loss of myocyte-expressed VEGF and initiate new capillary formation in response to repeated bouts of exercise?

Thus far, the only interventional study to approach the question of VEGF’s importance in the skeletal muscle capillary adaptation to exercise training reports partial inhibition in rats administered the VEGF receptor tyrosine kinase inhibitor (i.e., ZD-4190) (37). In that study, however, the rats given ZD-4190 were also subjected to bilateral occlusion of the femoral arteries (a model of peripheral arterial disease); therefore, the reported outcome reflects responses to muscle ischemia, in addition to exercise training, and not simply the role of VEGF under normal training conditions (37). Thus the importance of VEGF in regulating exercise-induced skeletal muscle angiogenesis under physiological conditions still remains unstudied.

The purpose of the present study was to determine whether skeletal muscle capillary adaptation to exercise training would occur in mice that do not express VEGF in myocytes. Accordingly, we report the effects of 6 wk of treadmill exercise training on skeletal muscle capillarity in myocyte-specific VEGF gene-deleted (mVEGF−/−) mice. We hypothesized that, if myocytes are an essential source of VEGF, then skeletal muscle capillary adaptation would not occur in response to exercise training in mVEGF−/− mice. Furthermore, we examined whether other well-known training-induced skeletal muscle adaptations, i.e., increased muscle oxidative and glycolytic enzyme activity levels, would still occur in mVEGF−/− mice and functionally compensate for the loss of muscle VEGF and contribute to exercise performance.

MATERIALS AND METHODS

This study was approved by the University of California, San Diego, Animal Care and Use Committee and conducted in accordance with all applicable local, state, and federal laws and institutional guidelines for the care and use of laboratory animals.
with guidelines outlined by the Guide for the Care and Use of Laboratory Animals (National Research Council). Mice were housed three to four animals per cage in a pathogen-free vivarium maintained on a 12:12 h day-night cycle and were provided standard chow (Harlan Tekland 8604, Madison, WI) and tap water ad libitum. At the conclusion of the experiments, mice were killed by surgical removal of the heart while under deep anesthesia (pentobarbital sodium, 60 mg/kg ip).

mVEGF$^{-/-}$ mouse model. The engineering of mVEGF$^{-/-}$ mice has previously been described (48). In brief, VEGFLoxP mice (background strain C57BL/6) kindly provided by Dr. Napoleon Ferrara, Genentech, San Francisco, CA) (13) were genetically crossed with transgenic mice (background strain C57BL/6J) expressing Cre recombinase, under the control of the muscle creatine kinase (MCK) promoter, i.e., myocyte-specific MCK-Cre mice (8). Expression of Cre recombinase in VEGF-LoxP mice inactivates all isoforms of the VEGF-A gene. Genotyping was performed on DNA extracted (DNeasy Tissue Kit; Qiagen, Valencia, CA) from mouse tail sections and later verified using skeletal muscle tissue. PCR analysis was performed using TaqPro Red Complete DNA Polymerase Master Mix (Denville Scientific, Metuchen, NJ) with the following probes: for VEGF forward primer 5'-TGGCAAGTTGAATAACCG-3' and reverse primer 5'-CTAGAGCCTGTTTCTAGG-3'. PCR analysis was performed using TaqPro Red Complete DNA Polymerase Master Mix (Denville Scientific, Metuchen, NJ) with the following probes: for VEGF forward primer 5'-TGGCAAGTTGAATAACCG-3' and reverse primer 5'-CTAGAGCCTGTTTCTAGG-3'.

Animal groups and data collection. For this study, 16 [n = 8 mVEGF$^{-/-}$, n = 8 wild type (WT)] mice were exercise trained on a rodent treadmill for 6 wk (details provided below). Data shown for sedentary (untrained) mice in this study have been previously reported (48). Because measurement of muscle structural variables required terminal tissue sampling, we could not perform a repeated-measures design before and after training within the same animal. Therefore, pretraining (sedentary) control data for the capillary-to-fiber ratio, and molecular and biochemical analysis (described below), and the second, gastrocnemius muscle was sliced in half at the muscle midbelly and quickly flash-frozen with freezing medium gel (TBS Tissue Freezing Medium; Triangle Biomedical Sciences, Durham, NC) in a liquid N$_2$-cooled isopentane bath. Muscle samples were stored at −80°C. For muscle morphometry, the gastrocnemius muscle was cut into serial 9-μm transverse sections and stained for capillarity using the alkaline phosphatase capillary staining method (46).

Stained muscle sections were viewed by light microscopy and digitally imaged at ×40 magnification by a researcher blinded to the experimental group. Muscle capillarity and fiber characteristics were assessed from deep and superficial regions within the lateral and medial gastrocnemius. Deep and superficial regions, as well as demarcation of medial and lateral muscle, were first determined from ×4 images and subsequently used to obtain ×40 images within each defined muscle region. Deep regions were defined as the inner half of the gastrocnemius muscle (closest in proximity to the plantaris and soleus muscles), whereas superficial regions were comprised of the outer half of the gastrocnemius (closest in proximity to the skin). Up to eight images were obtained from each quadrant in a pseudorandom selection process. Images were selected if they did not overlap with any previous image selected or did not violate the boundary used to demarcate the quadrant being imaged. The imaging technician was instructed not to “frame” each image per se but rather accept the random image position (after moving the microscope stage) within each quadrant, provided they did not violate the image selection criteria above. This resulted in an average of 935 ± 152 (SD) muscle fibers being analyzed from each animal. Within each image, mean fiber area was calculated from the total muscle area (excluding spaces between muscle fibers and any nonmuscle structures) using computer software (MATLAB version 7.0; The MathWorks, Natick, MA) divided by the total number of fibers. Nonmuscle areas were distinguished using an algorithm designed to differentiate color differences (RGB spectral analysis) between muscle and nonmuscle tissue.

VEGF enzyme-linked immunoassay. One-half of the gastrocnemius muscle (including both medial and lateral portions) was homogenized in a lysis buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.5% Triton X-100, and protease inhibitors (Complete Tablet; Roche, Mannheim, Germany). Homogenates were centrifuged at 4°C, 10,000 rpm, for 10 min, and supernatants were assayed for total protein concentration (Bio-Rad DC protein assay; Bio-Rad Laboratories, Hercules, CA). VEGF protein levels were measured using an enzyme-linked immunosassay kit for mouse (VEGF Mouse ELISA kit no. QIA52; Calbiochem, La Jolla, CA) designed for detecting VEGF164 and VEGF120 isoforms of VEGF-A. VEGF receptor expression was measured using a commercially available ELISA kit for VEGF-R1 and VEGF-R2 (nos. MVR100 and MVR200B, respectively; R&D Systems) according to the manufacturer’s specifications. The remain-
ing portion of the gastrocnemius muscle was used to measure muscle metabolic enzyme activities.

**Skeletal muscle metabolic enzyme activities.** All enzyme measurements were performed at room temperature with a Beckman model DU 640B spectrophotometer. To assay citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (β-HAD) activities, whole muscle homogenates (6–10 mg) were homogenized in 100 volumes (wt/vol) of buffer (175 mM KCl, 2 mM EDTA; pH 7.4) using a Polytron (PT1200E) homogenizer, subjected to three freeze/thaw cycles using liquid N₂, then centrifuged at 10,000 rpm for 1 min, and supernatant was collected. CS activity was assayed according to the method of Sere (55). β-HAD activity was assayed as per Bergmeyer (6) by following the oxidation of NADH spectrophotometrically. Phosphofructokinase (PFK) activity was measured in muscle homogenates by the method of Lowry et al. (38).

**Statistics.** Data shown are means ± SE. Individual effects of training (i.e., trained vs. sedentary) and genotype (i.e., WT vs. mVEGF) on capillary, VEGF, and enzyme activity measurements were determined using a 2 × 2 factorial ANOVA (i.e., genotype × training). When a main effect was observed, post hoc testing with an unpaired Student’s *t*-test was used to examine within-group differences. A mixed-model repeated-measures ANOVA was used to analyze the variables (body mass, maximal running speed, and endurance run times) measured in the same animal before and after training. Animals were the random factor, and training and genotype were fixed factors in the repeated-measure analyses. All statistics were performed using the Statview Statistical Software package (version 5.0.01; SAS Institute, Cary, NC). In all cases, significance was accepted at *P* < 0.05.

**RESULTS**

**Skeletal muscle VEGF expression.** Basal expression of VEGF protein in gastrocnemius muscle of exercised trained mVEGF⁻/⁻ mice were 95% lower (*p* < 0.05) compared with WT mice (Fig. 1). This finding is similar to that previously shown in sedentary mVEGF⁻/⁻ mice (48). Basal expression for both VEGF-R1 (or Flt-1) and VEGF-R2 (or Flk-1) was also significantly decreased (VEGF-R1 = 55% decrease, *P* < 0.01; VEGF-R2 = 64% decrease, *P* < 0.01) in the gastrocnemius muscle of exercise-trained mVEGF⁻/⁻ compared with trained WT mice (Fig. 1). These data are also similar to that previously shown in sedentary mVEGF⁻/⁻ mice, which exhibited a 63 and 69% decrease in VEGF-R1 and VEGF-R2, respectively, in mVEGF⁻/⁻ compared with WT mice (48).

**Training effects on skeletal muscle capillarity.** Histological assessment of muscle capillarity in deep (oxidative muscle fibers) vs. superficial (glycolytic muscle fibers) regions of the gastrocnemius muscle revealed differential capillary adaptation responses to training. In WT mice, exercise training significantly increased (by 59%) capillary density (ANOVA = genotype *P* < 0.001, training *P* = 0.01, interaction *P* = 0.038) in the deep portion of the gastrocnemius muscle (Fig. 2, A and B). A similar trend (33% increase) was also seen in the capillary-to-fiber ratio (ANOVA = genotype *P* < 0.01, training *P* = 0.13, interaction *P* = 0.08) in the deep portion of the gastrocnemius muscle in WT mice (Fig. 2A). As evident in Fig. 2, training produced no changes in gastrocnemius muscle capillarity (in either deep or superficial muscle regions) in mVEGF⁻/⁻ mice. Muscle fiber cross-sectional area was not affected by training in either WT or mVEGF⁻/⁻ mice (ANOVA = genotype *P* = 0.64, training *P* = 0.09, interaction *P* = 0.93; Fig. 2B). Gastrocnemius muscle capillarity in mVEGF⁻/⁻ mice posttraining remained significantly lower compared with WT mice (*P* < 0.05), similar to that previously reported in untrained mVEGF⁻/⁻ mice.

**Training effects on exercise performance.** As shown in Fig. 3, aerobic training significantly increased maximal running speed by 20% (Fig. 3A) and time to exhaustion by 13% (Fig. 3B) in WT mice. Training did not improve exercise performance in mVEGF⁻/⁻ mice.

**Body and muscle mass.** Body mass was 20% lower in mVEGF⁻/⁻ mice compared with WT before training (*P* < 0.05). Body mass increased in similarly mVEGF⁻/⁻ and WT
Fig. 2. A: representative images showing alkaline phosphatase-stained skeletal muscle sections from the deep gastrocnemius muscle regions of WT and mVEGF/−/− mice. Images shown are transverse sections magnified at ×40. B: comparison of capillary-to-fiber ratio, capillary density (no. of capillaries/mm²), and fiber cross-sectional area (FCSA, μm²) in deep and superficial regions of gastrocnemius muscle in sedentary and 6-wk exercise-trained mice. Shaded bars, data from mVEGF/−/− mice. Open bars, data from control (WT) mice. Data are means ± SE; n = 5–6 mice/group. *P < 0.05, significant difference compared with sedentary mice within same genotype (*) and significantly different compared with WT (†). Data for sedentary mice come from previously published work (48).
Exercise conditions for studying VEGF in locomotor skeletal muscle. In our mouse model, deletion of VEGF in striated muscle was achieved using the MCK promoter to drive Cre expression. We have previously reported evidence of reduced skeletal and cardiac muscle capillarity in untrained mVEGF−/− mice (48). Accordingly, we cannot exclude the possibility that impaired cardiac or skeletal muscle function, or both, might contribute to the reduction in exercise performance seen in mVEGF−/− mice. However, in the context of adaptation to exercise training, our study was designed to provide equivalent exercise stimuli to mVEGF−/− and WT mice (with normal VEGF levels) (Fig. 1) to allow us to address the functional importance of myocyte VEGF for exercise-induced angiogenesis in skeletal muscle. Under the training conditions we employed, the mVEGF−/− mice were exercised at an intensity level very near their maximum achievable level throughout the training regime.

Table 1. Demographic and hematological data before and after 6 wk treadmill training

<table>
<thead>
<tr>
<th></th>
<th>WT (n = 7)</th>
<th>mVEGF−/− (n = 8)</th>
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<tbody>
<tr>
<td><strong>Age, days</strong></td>
<td>Before training</td>
<td>92 ± 8</td>
</tr>
<tr>
<td><strong>Body mass, g</strong></td>
<td>After training</td>
<td>135 ± 9*</td>
</tr>
<tr>
<td><strong>Gastrocnemius muscle, mg</strong></td>
<td>NA</td>
<td>215 ± 13</td>
</tr>
<tr>
<td><strong>Gastrocnemius/body mass, %</strong></td>
<td>NA</td>
<td>25.1 ± 1.8*</td>
</tr>
<tr>
<td><strong>Heart, mg</strong></td>
<td>Before training</td>
<td>22.7 ± 1.0</td>
</tr>
<tr>
<td><strong>Heart/body mass, %</strong></td>
<td>After training</td>
<td>16.2 ± 0.7</td>
</tr>
<tr>
<td><strong>Hemoglobin, g/dl</strong></td>
<td>NA</td>
<td>13.4 ± 0.6</td>
</tr>
<tr>
<td><strong>Hematocrit, %</strong></td>
<td>NA</td>
<td>41 ± 1</td>
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Values are means ± SE; n, no. of mice. WT, wild type; mVEGF−/−, myocyte-specific vascular endothelial growth factor gene deleted. P < 0.05, significantly different compared with before training (same genotype) using repeated-measure ANOVA (*) and significantly different compared with WT using unpaired t-test analysis (†). NA, not able to measure before training (data on untrained mice have previously been published, see Ref. 48).

DISCUSSION

The principal finding in this study is that exercise-induced skeletal muscle angiogenesis requires paracrine expression of myocyte VEGF. Although several studies have implicated the importance of VEGF as a regulator of skeletal muscle angiogenesis, the significance of this work is that we have targeted VEGF gene inactivation to the myocyte population. Therefore, we are able to test the importance of myocyte-expressed VEGF to muscle structure and function in adult mice under physiological conditions. These data demonstrate that, 1) exercise-induced angiogenesis is prevented in the absence of myocyte-expressed VEGF and 2) nonmyocyte VEGF sources (e.g., endothelial cells, fibroblasts, macrophages, etc.) in the muscle or from other organs are not able to compensate for the loss of myocyte VEGF to stimulate the formation of new capillaries in response to exercise training.

Fig. 3. Comparison of maximal running speed (A) and submaximal endurance exercise capacity (B) in trained mVEGF−/− (n = 8) and WT (n = 7) mice before and after 6 wk aerobic exercise training. Values are means ± SE. *P < 0.05, significantly different compared with before training within the same genotype (†) and significantly different compared with WT, repeated-measures ANOVA (†).
Because WT mice were held to the same absolute training regime performed by the mVEGF−/− mice, the relative exercise intensity experienced by WT mice during training was much lower compared with mVEGF−/− mice. It is reasonable to predict that WT mice could have, and would have, attained greater muscular adaptations and much faster running speeds over the course of the training had they been exercised at an intensity near their maximum capacity. However, comparison of mice exercise trained at similar relative intensities would not have allowed us to exclude the possibility that the absence of a training-induced angiogenic response in the mVEGF−/− mice might have simply been due to a lower absolute exercise intensity (22, 23). The risk of our decision was that WT mice trained at the same absolute intensity as mVEGF−/− mice, and therefore a much lower relative intensity, would not receive a sufficient exercise stimulus to elicit a training adaptation. However, as evident in Fig. 2, this level of training did result in a greater number of capillaries in the deep region of the gastrocnemius in WT mice, indicating the training paradigm we used was sufficient to elicit skeletal muscle capillary adaptation. The fact that mVEGF−/− mice actually trained at a much higher relative intensity (and did not show capillary expansion) also supports the premise that there was sufficient exercise stimulus present to promote muscle capillary expansion in mVEGF−/− mice, if it could have occurred independent of myocyte-expressed VEGF.

When taking these data in context with another cell-specific VEGF gene-deleted mouse model (i.e., endothelial cell-specific VEGF knockout mice, VEGFΔEC KO) (35), it becomes evident that the cell type expressing VEGF likely plays an important role in its function. For example, VEGFΔEC KO mice demonstrate endothelial cell apoptosis and compromised vascular homeostasis that leads to sudden death in 55% of the mutant mice. However, the density of microvessels in several organs from VEGFΔEC KO mice were not altered (35). In contrast, our myocyte VEGF-deficient mice do not exhibit the same type of altered vascular homeostasis leading to early mortality but are unable to maintain normal capillary density in striated muscle (Fig. 2) (48, 56). Thus, although VEGF is capable of being secreted and transported throughout the body, it appears that the cellular source of VEGF and/or its microenvironment may be equally important for successful capillary maintenance and angiogenic signaling. This could, in part, also help explain the finding by Lloyd et al. (37) that VEGF receptor antagonism completely blocked skeletal muscle angiogenesis (collateral vessel enlargement) but only partially blocked muscle angiogenesis in response to training in their ischemic mouse model.

As previously seen in our sedentary mVEGF−/− mice (48), the expression of soluble VEGF receptors (R1 and R2) was significantly lower in exercise-trained mVEGF−/− mice compared with WT littermates (Fig. 1). It is worth noting that the magnitude decrease for both VEGF receptors in the exercise-trained mice is nearly identical that seen in our sedentary mice (i.e., VEGF-R1 sedentary 55% vs. trained 63% decrease; and VEGF-R2 sedentary 64% vs. trained 69% decrease). This could be viewed as evidence that the VEGF receptors themselves, albeit significantly reduced in mVEGF−/− compared with WT (P < 0.05), were most likely not responsible for the lack of skeletal muscle capillary adaptation to exercise we observed in the mVEGF−/−. On the other hand, it could be argued that reduced VEGF-R1 and VEGF-R2 expression due to the lack of VEGF could potentially be the reason for the lack of capillary adaptation. Although we cannot fully exclude the possibility that reduced VEGF receptor expression may have also contributed to the initial capillary rarefaction in sedentary mice and/or the lack of capillary adaptation in response to exercise training, it is evident from these data that the axis involving VEGF and its receptors is vitally important in the adaptive response of skeletal muscle microvessels to exercise.

Whereas these data provide evidence that myocyte expression of VEGF is essential for activity-induced skeletal muscle angiogenesis, potential limitations with our experimental design and gene knockout mouse model must also be recognized. First, although littermate sibling mice were used as controls for the data obtained in sedentary mice in our previous study (48), and in this study for the trained mice, the fact remains that data for sedentary and trained mice were not from the same litter. Thus concerns regarding the inherent variability of assays and/or variability from different batches of mice could question the interpretation of our data. However, we would note the magnitude of changes observed (between the sedentary and trained groups) in muscle capillarity, exercise performance, and enzyme activity in our WT mice are consistent with that seen in other training studies involving C57BL/6 mice (11, 14, 36, 42–44, 54, 59, 60). Moreover, we saw nearly identical...
reductions in VEGF (90% in sedentary and 95% in trained mice) and its receptors between sedentary (48) and trained (Fig. 1) groups. Based on the collective evidence involving this mouse model, we believe there is little cause for concern with the interpretability of data when comparing sedentary and trained groups. A second potential issue to note involves the creation of our mouse model. Because VEGF is inactivated during late embryogenesis [in conjunction with constitutive expression of MCK (57)], we cannot rule out the possibility that unforeseen developmental changes might also contribute to the absence of an exercise training-induced angiogenic response. For example, we find that metabolic enzymes, CS, β-HAD, and PFK (Fig. 4), are upregulated in untrained mVEGF−/− mice compared with the WT (discussed below). Therefore, one explanation may be that mVEGF−/− mice may have compensated to maximize O2 utilization, such that when challenged by exercise, no further increases are possible. However, even so, it is clear that this response could not maintain nor preserve normal exercise capacity observed in mVEGF−/− mice. Future studies to conditionally inactivate the myocyte VEGF gene in fully developed adult mice may help to further uncover potential compensatory changes that may have occurred in these life-long muscle VEGF-deficient mice.

Skeletal muscle enzymatic activity following exercise training. In general, exercise training is also expected to contribute to a greater aerobic capacity through mitochondrial and biochemical adaptations. For instance, exercise training leads to increases in the number and size of mitochondria, enhances oxidative and glycolytic enzyme activity levels, and results in a more efficient use of glucose and lipid stores (2, 15, 16, 27, 28, 53). In this study, we found that CS enzyme activity, which is one biomarker of oxidative metabolism expected to increase with training, was increased by only 25% in mVEGF−/− mice compared with a 45% increase in the WT mice (Fig. 4). Moreover, an increase in β-HAD activity was detected in WT, but not mVEGF−/−, trained mice. It should be noted that we have previously reported metabolic compensation (increased CS, β-HAD, and PFK activities) is already present in untrained (sedentary) mVEGF−/− mice (shown in Fig. 4) (48). Therefore, it is possible that the observed blunted oxidative enzyme response to exercise training is influenced by the already elevated baseline enzyme activity levels. In contrast to CS and β-HAD, mVEGF−/− mice were able to increase activity of the glycolytic enzyme PFK to the same extent as the WT group in response to exercise training (Fig. 4). Although we cannot provide a mechanistic explanation linking VEGF per se in the regulation of muscle metabolic enzymatic activity, collectively, these data suggest that mVEGF−/− mice may be attempting to maximize energy production in the face of losses in muscle capillarity. However, it is important to emphasize that the broad implications of selective regulation of glucose or lipid metabolism cannot be directly inferred solely from these enzymatic data. Of note, however, elevated skeletal muscle enzymatic activities have been reported in association with peripheral arterial disease and chronic obstructive pulmonary disease (9, 32, 39), and both conditions have also been reported to have lower or altered VEGF expression in skeletal muscle (3, 4, 58).

Last, because there is evidence that the muscle-capillary interface is a critical determinant for O2 transport (12, 17, 18, 29) and that muscle O2 conductance (and consequently muscle VO2) is dependent on skeletal muscle capillarity (5, 25, 26, 30, 31), it could be hypothesized that a lack of capillary adaptation to training contributed to the impaired exercise performance. Corroborating evidence comes from untrained thrombospondin-1 knockout mice, which have elevated skeletal muscle capillarity (and normal cardiac function) compared with WT mice and display a significant increase in exercise capacity (40). However, we cannot differentiate between central and peripheral limitation in mVEGF−/− mice, because impaired cardiac and/or ventilator function (due to reduced diaphragm muscle capillarity) may have also contributed to the reduced exercise performance.

Perspectives and Significance

These data provide evidence that myocyte-expressed VEGF is required for the muscle capillary adaptation to exercise training. Elevated muscle oxidative and glycolytic enzyme activities following training provide evidence that nonvascular skeletal muscle adaptations to training persist in the absence of myocyte VEGF expression. In particular, loss of myocyte VEGF may lead to greater reliance on glycolysis. However, it is also evident from these data that training-induced metabolic adaptations, seen in untrained and trained VEGF-deficient mice, by themselves are not sufficient to overcome the consequences of reduced muscle capillarity. These data demonstrate a vital role for myocyte VEGF in initiating the complex series of events required for exercise-induced skeletal muscle angiogenesis and provide additional evidence that skeletal muscle capillarity is a major contributing factor for overall exercise performance.

ACKNOWLEDGMENTS

We acknowledge the assistance of Patrick Giuliano for conducting the exercise training and muscle morphometric assessments and Kathleen Roberts for data analysis.

GRANTS

This research was supported by funds from the American Heart Association Western States Affiliate Grant no. 0365103Y (I. M. Olfert), Tobacco-Related Disease Research Program no. 14KT-0091 (I. M. Olfert), and by National Heart, Lung, and Blood Institute Grants FPG HL-091830 and R01 HL-84281 (P. D. Wagner, E. C. Breen). I. M. Olfert also received funding support as a Parker B. Francis pulmonary fellow.

DISCLOSURES

None.

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