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## Angiotensin II and VEGF Are Involved in Angiogenesis Induced by Short-Term Exercise Training

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### Abstract

Results from our laboratory have suggested a pathway involving angiotensin II type 1 (AT<sub>1</sub>) receptors and vascular endothelial growth factor (VEGF) in angiogenesis induced by electrical stimulation. The present study investigated if similar mechanisms underlie the angiogenesis induced by short-term exercise training. Seven days before training and throughout the training

period, male Sprague-Dawley rats received either captopril or losartan in their drinking water. Rats underwent a 3-day treadmill training protocol. The tibialis anterior and gastrocnemius muscles were harvested under anesthesia and lightly fixed in formalin (vessel density) or frozen in liquid nitrogen (VEGF expression). In controls, treadmill training resulted in a significant increase in vessel density in all muscles studied. However, the angiogenesis induced by exercise was completely blocked by either losartan or captopril. Western blot analysis showed that VEGF expression was increased in the exercised control group, and both losartan and captopril blocked this increase. The role of VEGF was directly confirmed using a VEGF-neutralizing antibody. These results confirm the role of angiotensin II and VEGF in angiogenesis induced by exercise.

regular aerobic exercise training can induce a series of adaptive responses in cardiovascular and skeletal systems. The adaptations of skeletal muscle include increases in the oxidative enzyme activity as well as the number and size of mitochondria. Skeletal muscle angiogenesis, determined by an increase in capillary density and/or capillary-to-fiber ratio, reflects a vital adaptation to exercise, contributing to the improved aerobic capacity ( $O_2$  transport,  $O_2$  conductance, and  $O_2$  extraction) of skeletal muscle. Although most of these results have been described after a long period of endurance training, results from our laboratory have suggested the possibility that the microcirculation of skeletal muscle can adapt and change structure very rapidly after exercise or electrical stimulation (2, 23, 26). The mechanisms underlying the capillary growth process in skeletal muscle are not yet understood. Different angiogenic factors have been characterized, and much attention has focused on vascular endothelial growth factor (VEGF). Several lines of evidence indicate that VEGF is involved in the regulation of the angiogenic process in vitro (25, 37) and in vivo (2, 8, 9, 19, 21, 29, 36). Accordingly, an elevation of VEGF mRNA has been reported in skeletal muscle after a single bout of exercise in rats (5, 11, 12) and humans (18, 28, 29). These studies suggest that the microcirculation of skeletal muscle is likely to respond in a more dynamic fashion than previously considered and may play an important role in short-term adaptations to exercise. Recently, results from our laboratory (2) have suggested a pathway involving angiotensin II (ANG II) type 1 ( $AT_1$ ) receptors and VEGF protein expression in angiogenesis induced by electrical stimulation. The present study investigated if these mechanisms underlie the angiogenesis induced by short-term exercise training.

## METHODS

The Institutional Animal Care Committee at the Medical College of Wisconsin and Marquette University approved all protocols and procedures. Seventy-four male Sprague-Dawley rats (8–9 wk old) were familiarized with treadmill running for 4 days before the beginning of training. This preliminary exercise was limited to 5–10 min. All animals were fed a standard laboratory rat chow (Purina) and water ad libitum. All animals were housed in single cages to permit assurance of proper daily fluid intake.

## Drugs

Captopril was obtained from Sigma (St. Louis, MO), losartan was obtained from Merck (West Point, PA), and monoclonal VEGF-neutralizing antibody was generously provided by Texas Biotechnology (Houston, TX).

## Experimental Design

After familiarization, age- and weight-matched rats were randomly assigned to the following four experimental protocols:

Experiment 1.

Eight rats were given water during the entire exercise protocol.

Experiment 2.

Eight rats were given captopril ( $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ) in their drinking water 7 days before and during the entire exercise protocol.

Experiment 3.

Eight rats were given losartan (50 mg/day) in their drinking water 7 days before and during the entire exercise protocol.

Experiment 4.

Thirteen rats were given VEGF-neutralizing antibody (0.6 mg/100 g ip) or PBS (control) 1 day before and the following 2 days of the exercise protocol.

Additionally, 8 animals receiving water, 8 animals receiving captopril, 8 animals receiving losartan, and 13 animals receiving VEGF-neutralizing antibody or PBS were used as sedentary controls.

## Training Protocol

All rats were assigned to run on a treadmill at 20 m/min, 5% grade, for 1 h/day for 3 days. Exercise began daily at 9:00 AM and was repeated for 3 consecutive days. Groups were randomly placed on the treadmill each day. After the last training period, all animals were anesthetized with pentobarbital sodium within 2 h of exercising [\(5\)](#). The hindlimb muscles were removed, and the animals were euthanized.

## Surgical Catheter Implantation and Acute Arterial Pressure Measurements in Rats Treated With Captopril

After 10 days of drug treatment, rats ( $n = 4$ ) were anesthetized with pentobarbitol sodium (50 mg/kg ip), a polyvinyl catheter was placed in the right carotid artery and right jugular vein, and blood pressure (BP) was measured for at least 15 min. The effectiveness of chronic inhibition of angiotensin-converting enzyme (ACE) by oral captopril was determined by recording the changes in arterial pressure induced by an intravenous bolus of angiotensin I (ANG I; 0.1 ml at 1 ng/ml iv). The effectiveness of chronic blockade of AT<sub>1</sub> receptors by oral losartan was previously determined by an intravenous bolus of ANG II (2).

## Tissue Harvest and Morphological Analysis of Vessel Density

After 3 days of exercise training, the animals were euthanized by an overdose of Beuthanasia solution (Sigma). The gastrocnemius (GA) and tibialis anterior (TA) muscles were removed, rinsed in physiological saline solution (PSS), and weighed. The GA was then separated into white (GAW) and red (GAR) parts. The separation of the red from the white muscle was performed after the whole GA muscle was harvested from the leg. The muscle was opened with a delicate scissor, and the red portion was removed from the white part of the GA muscle. A 100-mg section was taken from each muscle and immediately frozen in liquid nitrogen for Western blot analysis. The remaining tissues were lightly fixed overnight in 0.25% formalin solution. The fixed muscles were sectioned as previously described (22). From every animal, two slices of each muscle were made and immersed in a solution of 25 µg/ml rhodamine-labeled *Griffonia simplicifolia* I (GS-I) lectin (Sigma). After the 2-h exposure to GS-I lectin, the muscles were rinsed and mounted on microscope slides in a water-soluble mounting medium consisting of toluene and acrylic resin (SP ACCU-MOUNT 280, Baxter Scientific; McGaw Park, IL) (13).

The sections were visualized using a video fluorescent microscope system with epi-illumination (Olympus ULWD CD Plan, x20 objective). Ten representative fields were selected for study from each muscle slice. Images were digitized and quantified with automated computer vessel counting as previously described (30).

## Western Blot Analysis to Detect the Presence of VEGF Protein

One hundred milligrams of each muscle specimen were homogenized, and the protein was suspended in potassium buffer (10 mM). Five micrograms of protein (as determined by protein assay kit, Bio-Rad; Hercules, CA) from each muscle and a tumor cell line known to express VEGF at high levels (C<sub>6</sub>, American Type Culture Collection, 107-CCL) were separated on a 12% denaturing polyacrylamide gel. The gels were transferred to a nitrocellulose membrane, which was blocked for 2 h at room temperature in 5% nonfat dry milk diluted in Tris-buffered saline (50 mM Tris and 750 mM NaCl; pH = 8) with 0.08% Tween 20 (Bio-Rad). The blots were then incubated overnight with a polyclonal antibody to a peptide derived from the human VEGF sequence (1:1,000 dilution, clone G143-850, Pharmingen). Washed blots were then incubated with goat anti-mouse secondary antibody at a dilution of 1:1,000 for 1 h at room temperature

and then subjected to the SuperSignal West Dura chemiluminescence substrate (Pierce; Rockford, IL) detection system. Membranes were exposed to X-ray film (Fuji Medical; Stamford, CT) for 30~60 s and developed using a Kodak M35 X-Omat processor. For the quantitative VEGF analysis, film was always exposed for a period of time that ensured that all signals were within the linear range of the detection of the film. The VEGF band intensity was quantified using a Morphometry Imaging System (Metamorph, Universal Imaging), and values are expressed as a percentage of the C<sub>6</sub> tumor cell standard.

## VEGF-Neutralizing Antibody to Confirm the Role of VEGF in Angiogenesis

To determine whether VEGF was essential to angiogenesis induced by short-term exercise training, we conducted an additional protocol using two groups of rats (7 wk). Three doses of monoclonal VEGF-neutralizing antibody (Texas Biotechnology) were administered during the period of exercise training. The protocol for administration of VEGF-neutralizing antibody was modified from Zheng et al. (36), and the dose was based on our previous results (2). One day before the exercise protocol, 0.6 mg/100 g antibody was injected intraperitoneally in all rats. Just before the exercise was started, the rats received intraperitoneal injections on days 1 and 2. All rats were euthanized on day 3, within 2 h after the treadmill training was completed.

## Data Analysis and Statistics

For each muscle, the vessel counts of all the selected fields (10 scans × 2 slices for each muscle) were averaged to a single vessel density. Vessel density was expressed in terms of mean number of vessel-grid intersections per microscope field (0.224 mm<sup>2</sup>). For each experimental group, the measured vessel density of the muscles was compared with the same muscle from sedentary group. All values are presented as means ± SE. The significance of differences in values measured in all groups was evaluated using a two-factor ANOVA (drug × condition). Significant differences were further investigated using a post hoc test (Tukey's).

## RESULTS

Table 1 summarizes the body weights and muscle-to-body weight ratios of the control sedentary and exercised rats, those treated with captopril or losartan and also those treated with VEGF-neutralizing antibody. All rats were the same age (9 wk old) at the start of the experiments, and there were no significant effects of drug on body or muscle weights. Although body weight increased after 3 days of exercise, the muscle weights of all animals analyzed were not different.

Table 1. Body weights and GA and TA muscle weight-to-body weight ratios of exercised and sedentary rats

Groups	n	Body Weight		GA/Body Weight	TA/Body Weight
		Start weight	End weight		
Control exercise	8	249.0 ± 5.4	287.9 ± 3.5*	0.52 ± 0.03	0.20 ± 0.01

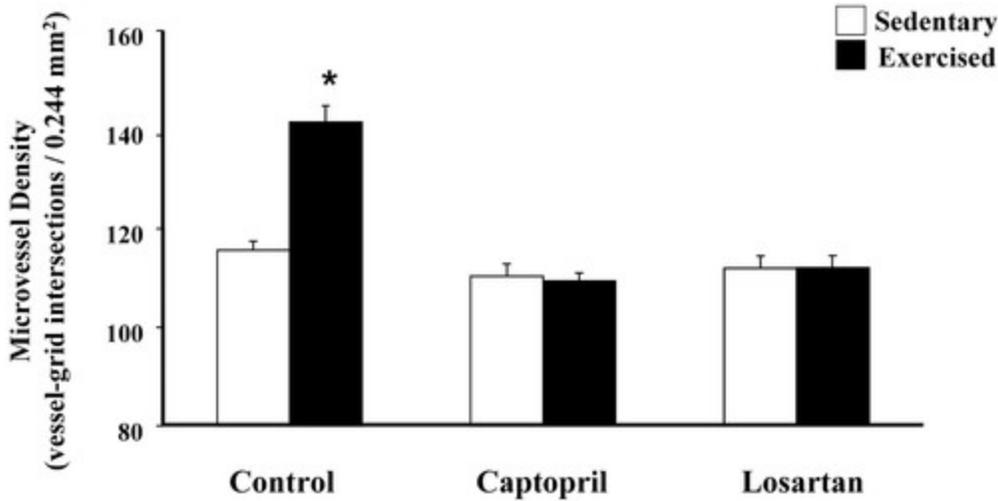
Groups	n	Body Weight		GA/Body Weight	TA/Body Weight
		Start weight	End weight		
Control sedentary	8	248.1 ± 4.6	277.9 ± 7.1*	0.57 ± 0.01	0.21 ± 0.01
Captopril exercise	8	249.8 ± 4.6	275.4 ± 3.3*	0.52 ± 0.04	0.20 ± 0.01
Captopril sedentary	8	246.5 ± 3.1	279.6 ± 2.8*	0.57 ± 0.01	0.21 ± 0.01
Losartan exercise	8	246.1 ± 5.0	278.5 ± 2.7*	0.57 ± 0.02	0.21 ± 0.01
Losartan sedentary	8	248.5 ± 6.3	283.8 ± 5.5*	0.56 ± 0.01	0.20 ± 0.01
Control (PBS) exercise	7	251.1 ± 7.34	258.9 ± 6.9*	0.55 ± 0.01	0.20 ± 0.01
Control (PBS) sedentary	7	233.3 ± 11.3	249.3 ± 12.8*	0.51 ± 0.01	0.19 ± 0.01
VEGF Ab exercise	6	223.8 ± 1.7	233.3 ± 1.5*	0.52 ± 0.01	0.20 ± 0.01
VEGF Ab sedentary	6	229.2 ± 3.6	247.8 ± 3.7*	0.53 ± 0.01	0.19 ± 0.01

Values are means ± SE; n = number of muscles in each group. Body weights are given in grams; muscle weights are given in milligrams per kilogram. GA, gastrocnemius muscle; TA, tibialis anterior muscle; Ab, vascular endothelial growth factor (VEGF)-neutralizing antibody.

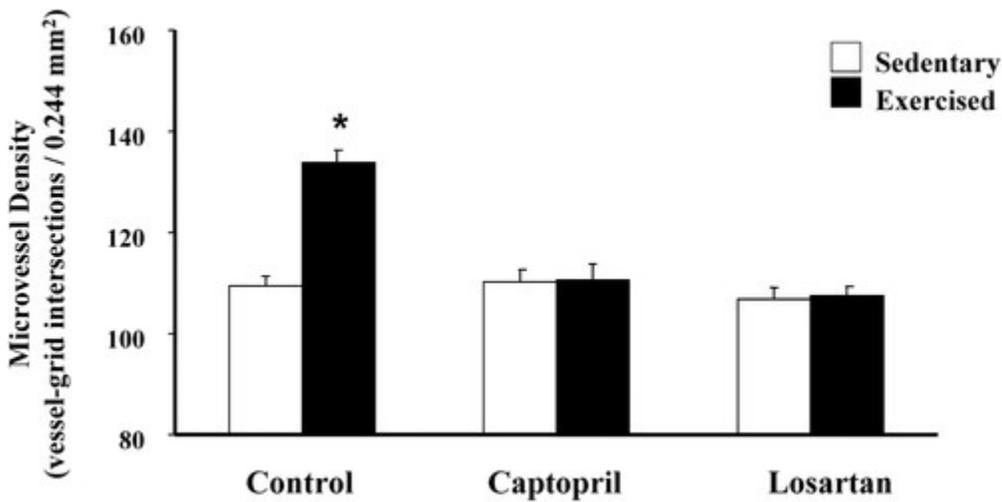
\*Significant versus before exercise,  $P < 0.05$ .

The effectiveness of the oral captopril dose used was determined by assessing the change in mean arterial pressure in response to a bolus dose of ANG I. There was a significant increase in mean arterial pressure ( $51 \pm 9$  mmHg) after an acute bolus of ANG I in the control group (data not shown). In contrast, the increase of the mean arterial pressure of animals treated with captopril ( $20 \pm 3$  mmHg) was significantly lower compared with that of control animals.

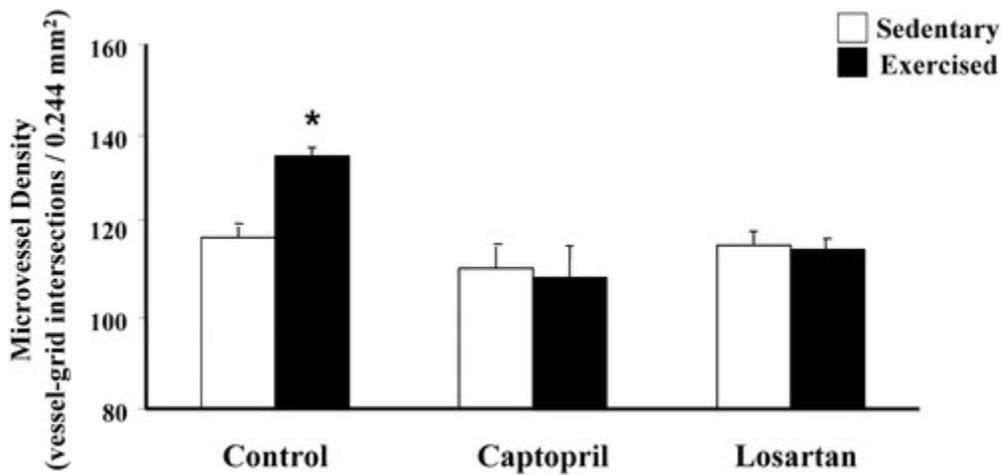
A significant increase in the vessel density of the control group was observed after 3 days of running in all muscles analyzed. As shown in Fig. 1, vessel density was increased by 23% in TA muscle ( $P < 0.05$ ). The increase of vessel density of TA muscle was completely blocked after 10 days of oral treatment with captopril ( $-0.68 \pm 1.54\%$ ,  $P < 0.05$ ) or losartan ( $0.33 \pm 2.49\%$ ,  $P < 0.05$ ). Figure 2 shows that vessel density in GAW muscle was increased after 3 days of exercise on treadmill (23%,  $P < 0.05$ ). Captopril and losartan also inhibited the increases in vessel density in GAW muscle ( $0.4 \pm 2.88$  and  $0.82 \pm 2.03\%$  for captopril and losartan, respectively,  $P > 0.05$ ). In the GAR muscle (Fig. 3), exercise caused an increase of 16% in vessel density ( $P < 0.05$ ). Both captopril and losartan blocked this response ( $-1.47 \pm 5.55$  and  $-0.48 \pm 2.02\%$  for captopril and losartan, respectively,  $P < 0.05$ ).



**Fig. 1.** Change in vessel density of the tibialis anterior (TA) muscle in sedentary ( $n = 8$ ) and exercise-trained rats ( $n = 8$ ) and those treated for 10 days with captopril ( $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ,  $n = 16$ ) and losartan ( $50 \text{ mg/day}$ ,  $n = 16$ ) after 3 days of exercise training. \*Significance vs. sedentary rat,  $P < 0.05$ .

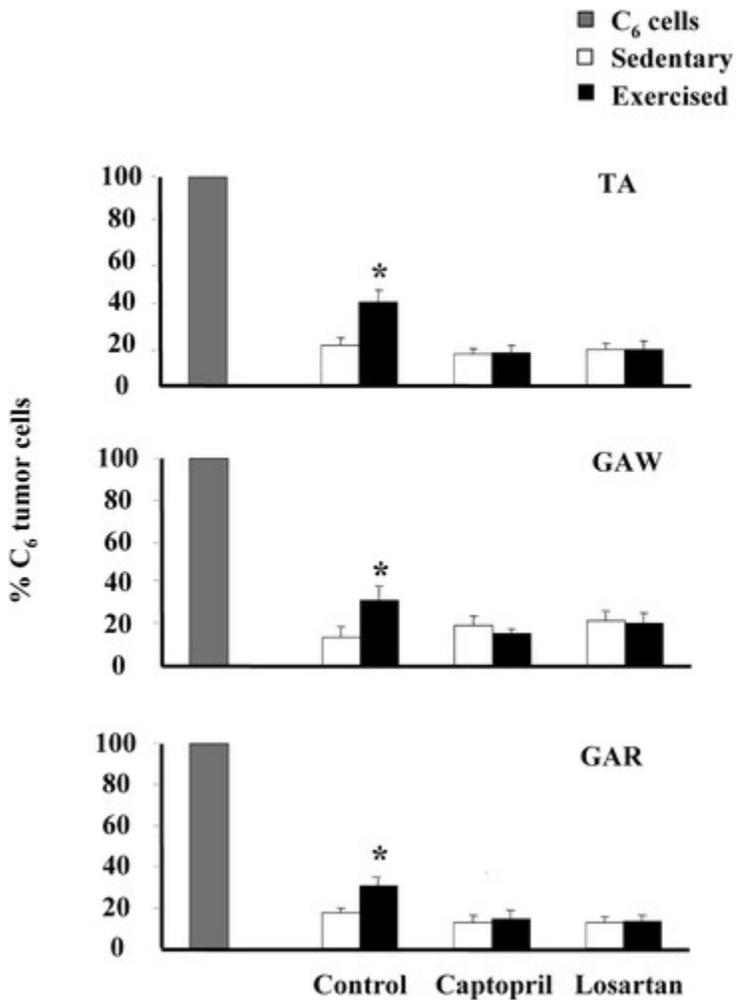


**Fig. 2.** Change in vessel density of the white part of the gastrocnemius (GAW) muscle in sedentary ( $n = 8$ ) and exercise-trained rats ( $n = 8$ ) and those treated for 10 days with captopril ( $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ,  $n = 16$ ) and losartan ( $50 \text{ mg/day}$ ,  $n = 16$ ) after 3 days of exercise training. \*Significance vs. sedentary rat,  $P < 0.05$ .



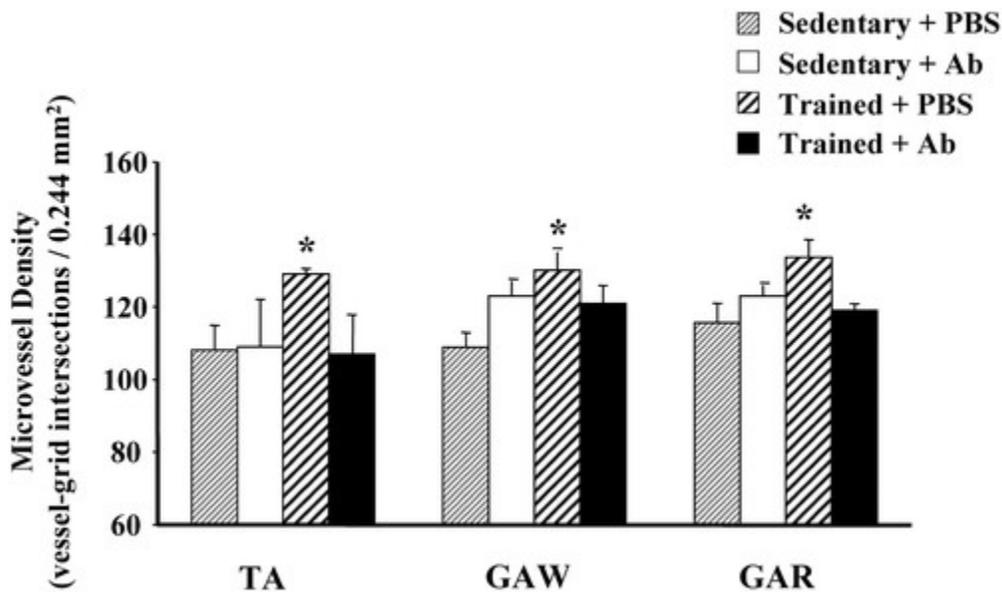
**Fig. 3.** Change in vessel density of the red part of the gastrocnemius (GAR) muscle in sedentary ( $n = 8$ ) and exercise-trained rats ( $n = 8$ ) and those treated for 10 days with captopril ( $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ,  $n = 16$ ) and losartan ( $50 \text{ mg/day}$ ,  $n = 16$ ) after 3 days of exercise training. \*Significance vs. sedentary rat,  $P < 0.05$ .

Western blot analysis and quantitative densitometry were used compare the responses of VEGF expression in the skeletal muscle after 3 days of exercise in all groups of animals (Fig.4). VEGF protein levels were significantly increased by 3 days of exercise in control animals in all muscles analyzed ( $P < 0.05$ ). This response was completely blocked or strongly attenuated by both captopril and losartan in all muscles studied.



**Fig. 4.** Quantitative densitometry of vascular endothelial growth factor (VEGF) protein in three different groups of rats: control (TA,  $n = 10$ ; GAW,  $n = 12$ ; GAR,  $n = 11$ ), captopril treated (TA,  $n = 13$ ; GAW,  $n = 13$ ; GAR,  $n = 10$ ), and losartan treated (TA,  $n = 13$ ; GAW,  $n = 10$ ; GAR,  $n = 12$ ). Values are expressed as a percentage of control (C<sub>6</sub> tumor cells). \*Significance vs. sedentary,  $P < 0.05$ .

To evaluate the role of VEGF in the angiogenic response, a group of rats was administered a monoclonal VEGF-neutralizing antibody during the exercise period. As shown in Fig. 5, the angiogenesis induced by exercise training was completely blocked in all muscles analyzed (sedentary + Ab vs. trained + Ab groups) after chronic treatment with a high dose of VEGF-neutralizing antibody when compared with trained rats treated with PBS (trained + PBS).



**Fig. 5.** Vessel density changes in skeletal muscle after 3 days of exercise training associated with treatment with monoclonal VEGF-neutralizing antibody (Ab). TA, GAW, and GAR muscles of control rats (PBS treated,  $n = 7$  sedentary and 7 trained) and those treated with monoclonal VEGF-neutralizing antibody (0.6 mg/kg 1 day before the protocol and on *days 1* and 2,  $n = 6$  sedentary and 6 trained) are shown. \*Significance vs. sedentary rats.

## DISCUSSION

The main finding of this study is that 3 days of aerobic exercise training significantly increased vessel density of both GAW and GAR muscles as well as TA muscle compared with sedentary control animals. Neither captopril nor losartan had any effect on vessel density or VEGF expression in sedentary rats. However, the drugs (captopril or losartan) blocked VEGF protein expression and the angiogenesis induced by short-term exercise training.

Increases in capillary density represent an essential adaptive response of skeletal muscle to repeated exercise, causing an increase in the number of capillaries per muscle fiber, which enhances  $O_2$  transport conductance between the microcirculation and mitochondria. It is well known that physiological angiogenesis is a compensatory response to prolonged imbalances between the metabolic requirements of the tissue and the perfusion capabilities of the vasculature (1). The mechanisms underlying the capillary growth process remain unknown. It is likely that a variety of factors are required to initiate physiological angiogenesis in response to exercise. On the basis of previous work (3, 21) *in vitro* and *in vivo*, reduced  $O_2$  tension as well as growth factors, hormones, and mechanical factors have been proposed to be important in the angiogenesis process in cardiac and skeletal muscle.

The extensive literature relating VEGF to vessel growth provides strong evidence that VEGF promotes angiogenesis in many tissues and under different conditions. VEGF, also known as vascular permeability factor, is the most potent and direct angiogenic factor known *in vitro* and *in vivo* (8, 10) with some specificity for vascular endothelial cells (22). Recent work from our laboratory (2) has shown that VEGF is partially responsible for the angiogenesis induced by

electrical stimulation in skeletal muscle. Results from the present study showed that only 3 days of exercise can cause increases in VEGF protein expression. Increases in VEGF mRNA have been shown as a consequence of electrical stimulation (3,19) and exercise (5, 11, 12, 18, 28, 29). Breen et al. (5) have shown that mRNA of angiogenic factors in skeletal muscle [VEGF, basic fibroblast growth factor (bFGF), and transforming growth factor (TGF)- $\beta_1$ ] increased after 1 h of exercise in intact animals. Accordingly, Richardson et al. (28) and Gustafsson et al. (18) found increases in VEGF (but not in TGF- $\beta$  or bFGF) mRNA after 45–60 min of leg-knee extensor exercise in humans; however, these changes in mRNA were not necessarily accompanied by changes in protein. It seems that VEGF may be involved in the early process of angiogenesis induced by exercise, because, after long periods of training, the amount of VEGF gene expression induced by exercise is attenuated (29). Because VEGF is upregulated after electrical stimulation and exercise, it has been considered a potent angiogenic factor; however, there is only one report (29) that measured both increases in VEGF gene expression and vessel density. The present study is the first to indicate that 3 days of exercise increases VEGF protein expression in skeletal muscle, with a consequent increase in capillary density. The present results also demonstrate that treatment with VEGF-neutralizing antibody completely blocked the angiogenesis induced by exercise compared with control groups. These responses, together with our previous results (2), provide strong support for the hypothesis that VEGF plays an important role in the angiogenesis induced by exercise. The results of the present work also suggest that angiogenesis may precede the changes in oxidative proteins, suggesting that the microvasculature of skeletal muscle is capable of undergoing rapid remodeling in response to exercise. This rapid increase in vessel density may account for the short-term (7 days) training effects previously reported when oxidative enzyme content is as yet unchanged (27).

The mechanisms underlying the change in VEGF expression as well as the capillary growth process are still unclear. Local hypoxia may be postulated as a trigger of increases in VEGF and angiogenesis induced by exercise. Although some experiments (4, 5, 15, 17, 18, 28,33) in vitro and in vivo have shown increases in VEGF under hypoxic conditions, the role of hypoxia in increasing VEGF mRNA during exercise is not completely understood. Breen et al. (5) showed that 1 h of exercise or hypoxia itself can independently increase VEGF gene expression in skeletal muscle and that the increase in expression was greater when the exercise was done under hypoxic conditions. More recently, Gustafsson et al. (18) found decreases in the  $P_{O_2}$  in working muscles after 30–45 min of leg-knee extension. This study also showed a correlation between increases in VEGF gene expression and increases in hypoxia-inducible factor mRNA. Conversely, Richardson et al. (28), using the same protocol of exercise (leg-knee extension), failed to demonstrate a correlation between cell  $P_{O_2}$  and VEGF mRNA. In that study (28), the increases in VEGF gene expression were of the same magnitude when the exercise was done in normoxic or hypoxic conditions. Some biomechanical events, such as shear stress, capillary wall tension, or vascular stretch, have been considered possible inducers of VEGF expression (21); however, these hypotheses remain unclear. While it has been shown that increases in blood flow can be responsible for releasing growth factors, Roca et al. (31) failed to demonstrate increases in VEGF mRNA expression in skeletal muscle of dogs during passive hyperperfusion. On the other hand, a recent report (37) showed that stretch induces VEGF gene expression and protein upregulation in coronary microvascular endothelial cells and cardiac myocytes in vitro. During exercise, high quantities of ATP are consumed, releasing inorganic

phosphate and contributing to increases in ADP and AMP, thus increasing levels of adenosine. Adenosine is a purine nucleoside composed of adenine and ribose joined by a glycosidic bond. Some evidence has shown that adenosine can be modulated by hypoxia (20) when the metabolic rate increases (14), stimulating endothelial proliferation in vitro as well as growth of blood vessels in in vivo models. These characteristics suggest an important regulatory role of adenosine in VEGF expression and consequently in angiogenesis. Recent evidence has shown that adenosine could increase VEGF mRNA in cultured cells (16, 17). Accordingly, Fischer et al. (10) reported that one inhibitor of cAMP phosphodiesterase (rolipram) completely blocked the upregulation of VEGF mRNA induced by adenosine. Results from Gu et al. (15) suggested that adenosine (acting by VEGF) can be considered as a maintenance factor for the vasculature, responsible for balancing the size of the capillary network according to the metabolic rate.

An interaction between the renin-angiotensin system (RAS), VEGF, and consequently angiogenesis has been suggested previously (6, 7, 24, 35). Results from our laboratory have shown that infusion of ANG II increases vessel density in skeletal muscle (24). Williams et al. (35) demonstrated that ANG II significantly increased VEGF gene expression, suggesting that besides changes in hemodynamics, ANG II could locally influence permeability and growth in the vascular endothelium. More recently, Chua et al. (6) demonstrated that ANG II was able to efficiently upregulate VEGF expression in cultured cells.

Otani et al. (25) showed that ANG II itself cannot increase either VEGF gene expression or cell proliferation; however, it can potentiate VEGF-dependent cell proliferation and tube formation of retinal microvascular endothelial cells through induction of the VEGF receptor KDR/Flk-1. Results from our laboratory (2) have suggested that the RAS is involved in VEGF protein expression and angiogenesis induced by electrical stimulation. The present study demonstrates that the induction of VEGF protein by exercise can also be modulated by the RAS, because treatment with captopril or losartan completely blocked the increased expression of VEGF.

The effects of ACE inhibitors on the growth pathway have been described in both in vitro and in vivo studies. Volpert et al. (34) showed that captopril inhibited bFGF-induced corneal neovascularization in female rats. This study also demonstrated that captopril inhibited cell migration in vitro. More recently, Gavin et al. (11) showed that the 4.8-fold increase in VEGF gene expression induced by exercise was not attenuated by acute injection of captopril; however, the drug decreased the expression of mRNA for the VEGF receptor KDR/Flk-1 but not Flt-1. Because KDR/Flk-1 is the VEGF receptor responsible for vasculogenesis (8, 9), this result agrees with the hypothesis that captopril can decrease vessel density induced by exercise. In agreement with Gavin et al. (11) and with this hypothesis, our results showed that captopril could significantly attenuate the angiogenesis induced by exercise. In addition, we were able to demonstrate that after chronic treatment with captopril (10 days), the VEGF protein increases in skeletal muscle induced by exercise were completely blocked. In our study, captopril did not have any effect on vessel density in sedentary or control animals.

The present study suggests a pathway where ANG II, acting through the AT<sub>1</sub> receptor, increases VEGF protein expression and consequently induces vessel growth. This hypothesis

was confirmed when the VEGF protein expression and angiogenesis were completely inhibited after treatment with ACE inhibitor or AT<sub>1</sub> receptor blocker. A recent report (32) has suggested a complimentary pathway, where VEGF can induce ACE mRNA in cultured human umbilical vein endothelial cells and thereby increase local ANG II production. Taken together, these results suggest that a synergy between VEGF and ACE might play an important mechanism underlying angiogenesis.

In summary, we confirmed the hypothesis that VEGF plays an important role in the angiogenesis induced by short-term exercise training and that the expression of this growth factor is partially controlled by the RAS. The effects described in this study should be considered during treatments in which the blood supply to tissues is impaired and neovascularization is a major therapeutic goal.

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## FOOTNOTES

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