Effect of short-term exercise training on angiogenic growth factor gene responses in rats

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Gavin, Timothy P., and Peter D. Wagner. Effect of short-term exercise training on angiogenic growth factor gene responses in rats. J Appl Physiol 90: 1219–1226, 2001.—We investigated whether 1) 5 days of exercise training would reduce the acute exercise-induced increase in skeletal muscle growth factor gene expression; and 2) reductions in the increase in growth factor gene expression in response to short-term exercise training would be coincident with increases in skeletal muscle oxidative potential. Female Wistar rats were used. Six groups (rest; exercise for 1–5 consecutive days) were used to measure the growth factor response through the early phases of an exercise training program. Vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TGF-β1), and basic fibroblast growth factor (bFGF) mRNA were analyzed from the left gastrocnemius by quantitative Northern blot. Citrate synthase activity was analyzed from the right gastrocnemius. VEGF and TGF-β1 mRNA increased after each of 5 days of exercise training, whereas exercise on any day did not increase bFGF mRNA. On day 1, the VEGF mRNA response was significantly greater than on days 2–5. However, the reduced increase in VEGF mRNA observed on days 2–5 was not coincident with increases in citrate synthase activity. These findings suggest that, in skeletal muscle, 1) VEGF and TGF-β1 mRNA are increased through 5 days of exercise training and 2) the reduced exercise-induced increase in VEGF mRNA responses on days 2–5 does not result from increases in oxidative potential.

vascular endothelial growth factor; transforming growth factor-β1; basic fibroblast growth factor; citrate synthase activity

REGULARLY PERFORMED ENDURANCE exercise induces major adaptations in skeletal muscle. These include training-induced changes in muscle substrate utilization, mitochondrial content, biochemical enzyme/protein activities, and capillarization (see Refs. 1, 6, 21, and 23 for review). In addition, despite extensive characterization of these training-induced changes, very little is known about the molecular events responsible for initiating and maintaining these adaptations.

Among the early gene responses are increases in various growth factors that are believed to be important regulators of exercise-induced skeletal muscle angiogenesis. In rats, a single, 1-h exercise bout [20 m/min, 10° incline, which represents 55% of maximal $O_2$ uptake ($\dot{V}O_2_{\text{max}}$); Ref. 7], produces rapid (increased immediately postexercise) and transient (returns to baseline 4 h postexercise) increases in mRNA of vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TGF-β1), and basic fibroblast growth factor (bFGF) (7). These exercise-induced growth factor gene responses are smaller in response to exercise of lower intensity (15 min, 10° incline), consistent with reports that exercise intensity can influence exercise-induced angiogenesis (16, 12). In addition, the increases in VEGF and TGF-β1 mRNA are greater in response to hypoxic exercise (7). Chandel et al. (8) have shown that the hypoxic activation of VEGF transcription may be regulated via a mitochondrial-dependent signaling process involving reactive oxygen species (ROS).

In skeletal muscle, there is an intimate relationship between capillarity and metabolic requirements. It is from this relationship that the theory of metabolically regulated exercise-induced angiogenesis arises. In this theory, prolonged imbalances between the perfusion capabilities of the blood vessels and the metabolic requirements of the tissue cells lead to modification of the vasculature to satisfy the tissue needs (1). In human skeletal muscle, the VEGF response to acute exercise is attenuated concomitant with increases in skeletal muscle aerobic capacity, oxidative potential, and capillarity after 8 wk of endurance exercise training, suggesting that VEGF expression is subject to a negative feedback mechanism as exercise adaptations occur (32). Relatively recently, evidence has been reported that some training adaptations may occur already within 1–2 wk after the commencement of an exercise training program. Short-term exercise training can produce increases in mitochondrial potential (9, 28, 29, 39). Starrrit et al. (39) recently demonstrated that citrate synthase activity (CSA) increases within the first 5 days of exercise training, whereas mitochondrial ATP production rate is increased within 10 days. In rats, a single acute treadmill exercise bout can produce a transient increase in citrate synthase mRNA, whereas 1 wk of exercise training can produce a 30% increase in CSA (29). After short-term exercise training, exercise...

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at the same contractile activity results in a smaller decrease in high-energy phosphates, smaller increases in P₄ and creatine, slower glycogen depletion, lower lactate production, and a greater reliance on fat oxidation for energy at the same contractile activity (9, 13–15, 37). These exercise-induced adaptations are beneficial in that subsequent exercise bouts result in smaller disturbances in intracellular homeostasis.

The purpose of this study was to investigate the effects of a short-term exercise training program on the angiogenic growth factor response to exercise. First, we determined the acute exercise intensity that produced the greatest increase in angiogenic growth factors (15, 20, or 25 m/min at 10° incline). Second, using this exercise intensity, we exercised animals for 5 consecutive days. We hypothesized that 1) 5 days of exercise training would produce consistent increases in angiogenic growth factor responses and 2) if a reduction in the exercise-induced increase in growth factor mRNA response was observed during the training program, this reduced response would be related to changes in oxidative potential. We demonstrate here that 1) VEGF and TGF-β₁ are consistently increased during 5 consecutive days of exercise training; 2) the VEGF mRNA response to exercise is greatest after exercise on day 1; and 3) the reduction in the exercise-induced increase in VEGF mRNA responses to exercise on days 2–5 is not related to increases in oxidative potential. We hypothesized that 1) VEGF and TGF-β₁ are consistently increased during 5 consecutive days of exercise training; 2) the VEGF mRNA response to exercise is greatest after exercise on day 1; and 3) the reduction in the exercise-induced increase in VEGF mRNA responses to exercise on days 2–5 is not related to increases in oxidative potential.

METHODS

This study was approved by the University of California, San Diego, Animal Subjects Committee. Female Wistar rats were used throughout the study. Age was 64 ± 2 days, and weight was 199 ± 12 g (means ± SD). All rats were first familiarized with a rodent treadmill (Omnipacer model LC-4, Omnicite, Columbus, OH) and taught to run at 20 m/min, 10° incline for 5 min, 48 h before the experimental protocol. At 10° inclination, the maximal treadmill running speed sustained for 2 min during an incremental maximal test for rats of this age, weight, sex, and strain is 40 m/min (unpublished observations). Animals were housed in their cages and allowed standard rat food and water ad libitum throughout the study.

Two separate protocols were used, with different groups of rats. In protocol A, to investigate the effect of exercise intensity on the angiogenic growth factor response to exercise, rats were randomly assigned into four groups (n = 6 per group): group 1 was kept at rest and groups 2–4 performed 1 h of treadmill running at either 15, 20, or 25 m/min, 10° incline, respectively. Exercise at 20 m/min and 10° incline represents 55% of \( V_{\text{O}_2\text{max}} \) in similar animals (7). In protocol B, to investigate the angiogenic growth factor response to 5 days of consecutive exercise, rats were randomly assigned into six groups (n = 6 per group): group 1 remained at rest and groups 2–6 performed 1 h of treadmill running at 20 m/min, 10° incline, for either 1, 2, 3, 4, or 5 consecutive days, respectively.

After completion of the final exercise bout, all animals were anesthetized with 2% halothane in \( O_2 \), the left gastrocnemius muscles (both heads) were removed, and total cellular RNA was isolated. Muscle samples were removed within 20 min after the completion of exercise. In protocol B, the right gastrocnemius muscles were also removed for the measurement of CSA. Samples were stored at −80°C until analysis.

RNA isolation and Northern analysis. The methods used for RNA isolation from rat gastrocnemius muscles and Northern blotting for VEGF, TGF-β₁, and bFGF have been described in detail previously (7). Briefly, total cellular RNA was isolated and separated by electrophoresis in a 6.6% formaldehyde-1% agarose gel. Fractionated RNA was transferred by Northern blot to Zeta-probe membrane (Bio-Rad, Hercules, CA), cross-linked to the membrane by ultraviolet irradiation for 1 min, and stored at 4°C. The blots were then probed with oligolabeled [α-\( ^32P \)] deoxyctydine triphosphate cDNA probes for VEGF, TGF-β₁, and bFGF. Prehybridization and hybridization were performed in 50% formamide, 5× saline sodium citrate (SSC), 10× Denhardt’s solution, 50 mM sodium phosphate, 1% SDS, and 250 μg/ml salmon sperm DNA at 42°C. Blots were washed with 2× SSC and 0.1% SDS at room temperature and 0.1× SSC and 0.1% SDS at 55°C (bFGF and TGF-β₁) or 65°C (VEGF). Blots were exposed to XAR-5 X-ray film (Eastman Kodak, New Haven, CT) by use of a Cronex Lightning Plus screen at −80°C. Autoradiographs were quantitated by densitometry within the linear range of signals and normalized to ribosomal 18S RNA levels.

CSA. CSA from the right gastrocnemius was determined spectrophotometrically at 30°C by the method of Srere (38). To prevent sampling bias and to be consistent in treatment with the homogenized tissue used for RNA analysis, the whole muscle was pulverized under liquid nitrogen, and 0.1 g was homogenized and sonicated at 4°C. The homogenate was frozen overnight at −20°C and rehomogenized and resoni-cated at 4°C. The samples were stored at −20°C until analysis (1 wk). The homogenate was diluted in an EDTA and EGTA phosphate buffer to a convenient concentration. Spectrophotometry was performed in Tris buffer. The acetyl-coenzyme A reagent was in the form of a sodium salt (Sigma Chemical, St. Louis, MO). The homogenate from each sample was assayed in triplicate.

Statistical treatment. Quantitative densitometry was used to measure the mRNA levels for VEGF, TGF-β₁, and bFGF. Lane loading variation was controlled for by normalization of growth factor densitometric signals with the ribosomal 18S RNA. A one-way analysis of variance was used to determine changes in mRNA (protocol A: exercise intensity or protocol B: day of exercise training) and CSA (protocol B). Fisher’s least significant differences test was used to determine significance between conditions. In protocol B, one sample was lost during mRNA processing from each of the rest, 1-day, 2-day, and 4-day groups. These samples were not replaced. Significance was established at \( P \leq 0.05 \) for all statistical sets, and reported data are means ± SE.

RESULTS

Protocol A. Figure 1 shows representative Northern blots in which VEGF (A), TGF-β₁ (B), and bFGF (C) mRNA levels were examined after the single, 1-h sub-maximal exercise runs at 15, 20, and 25 m/min. It is clear that VEGF mRNA increased with increasing exercise intensity between 15 and 20 m/min but was not further increased at 25 m/min. Figure 2 portrays the quantitative densitometry for VEGF (A), TGF-β₁ (B), and bFGF (C) mRNA normalized to 18S ribosomal RNA. Figure 2A demonstrates that exercise increases VEGF mRNA at all treadmill speeds (rest: 1.0 ± 0.1; 15 m/min: 2.9 ± 0.2; 20 m/min: 5.0 ± 0.7; 25 m/min: 4.5 ±
0.6). However, in contrast to the exercise intensity increases observed between 15 and 20 m/min, an increase from 20 to 25 m/min did not further increase VEGF mRNA. The TGF-β1 mRNA response to increasing exercise intensity is displayed in Fig. 2B. Exercise intensity does increase TGF-β1 mRNA, with statistically significant increases at 20 and 25 m/min (rest: 1.0 ± 0.1; 15 m/min: 1.3 ± 0.2; 20 m/min: 2.1 ± 0.1; 25 m/min: 2.0 ± 0.1). Similar to the VEGF mRNA results, TGF-β1 mRNA was not further increased by exercise at 25 m/min. A small but significant increase in bFGF mRNA was observed only with running at 20 m/min (rest: 1.0 ± 0.1; 15 m/min: 1.1 ± 0.1; 20 m/min: 1.3 ± 0.1; 25 m/min: 1.1 ± 0.1).

Protocol B. Figure 3 shows representative Northern blots for VEGF (A), TGF-β1 (B), and bFGF (C) mRNA levels examined at rest and after 1 h of submaximal running for 1–5 days of consecutive exercise training. It is clear that VEGF mRNA is increased by exercise after each day of the exercise training program and is greatest after the first day. In contrast, the increase in TGF-β1 mRNA is unchanged after exercise on days 1–5. We observed no increase in bFGF mRNA with exercise training in this protocol.

Figure 4 portrays the quantitative densitometry for VEGF (A), TGF-β1 (B), and bFGF (C) mRNA normalized to 18S ribosomal RNA. Exercise increases VEGF mRNA after each of 5 consecutive days of exercise training, with the largest increase observed after exercise on the first day (rest: 1.0 ± 0.1; day 1: 4.9 ± 1.2; days 2–5 mean: 2.9 ± 0.3) (Fig. 4A). We observed a small but consistent increase in TGF-β1 mRNA after exercise throughout the 5-day exercise training program (rest: 1.0 ± 0.1; days 1–5 mean: 1.7 ± 0.2) (Fig. 4B). In this protocol, there was no increase in bFGF mRNA (Fig. 4C). There was no significant difference in 18S rRNA throughout the training program (range: 79.2 ± 8.6 to 109.6 ± 14.8 arbitrary units).

CSA data are presented in Fig. 5. It is clear that CSA did not change during the 5 days of exercise training in our protocol. There were no significant correlations between CSA and VEGF mRNA (r = 0.15, P = 0.41), TGF-β1 mRNA (r = 0.19, P = 0.34), or bFGF mRNA (r = 0.08, P = 0.67).

**DISCUSSION**

The principal findings of the present study are 1) VEGF and TGF-β1 mRNA are increased after each of 5 consecutive days of exercise training; 2) the exercise-induced increase in the VEGF mRNA response after days 2–5 of exercise training was smaller than after day 1; and 3) differences in growth factor gene expression through the first 5 days of an exercise training program do not correlate with differences in oxidative potential as assessed by CSA. These results demonstrate that increases observed after a single acute exercise bout are not unique to the initial exercise bout but are observed throughout the first 5 days of an exercise training program.

**Exercise intensity and growth factor responses.** Previously, the exercise-induced responses in growth factor gene expression were shown to be sensitive to reductions in exercise intensity (7). Our results reiterate that VEGF, TGF-β1, and bFGF are sensitive to reductions in work intensities from 20 to 15 m/min; however, we did not see corresponding increases in gene expression when the workload was increased similarly from 20 to 25 m/min (Figs. 1 and 2). The observation that increases in exercise-intensity can produce increases in angiogenic growth factor responses is consistent with previous reports that exercise intensity can influence the quantitative increase in exercise-induced angiogenesis (EIA). Although it is difficult to compare increases in capillarization across studies due to differences in training duration and animal models, Gute and colleagues (18, 19) performed two separate training studies using the same analytic techniques and the same strain of rats. In analyzing the data from Gute and colleagues and calculating a weighted average of capillary-to-fiber (C/F) ratio based on the estimated fiber mass of red, white, and mixed portions of
the rat gastrocnemius (4), endurance training defined by the authors as low intensity (10–12 wk, 30 m/min, 0% incline, 60 min/day) increased the weighted C/F 10%, whereas endurance training defined by the authors as high intensity (12–14 wk, 32 m/min, 15% incline, 90 min/day) increased the weighted C/F 34%. From this analysis, it can be concluded that exercise intensity can influence EIA.

As exercise intensity increases, blood flow and muscle fiber recruitment also increase. In the red and mixed portions of the rat gastrocnemius, there is a linear increase in blood flow as treadmill speed increases, whereas in the gastrocnemius white portion blood flow increases only at or near maximal treadmill speed (27). As treadmill speed increases, there is a progressive recruitment of more peripheral fibers with lower oxidative capacities, such that blood flow distributions within muscles of the rat appear to be related to the recruitment pattern during locomotion (3). Therefore, increases in treadmill speed will result in progressive increases in the muscle mass being activated. If all muscle fibers can produce VEGF relatively equally, then this should result in progressive increases in VEGF mRNA with increases in treadmill speed. Consistent with this, VEGF mRNA does increase when treadmill speed is increased from 15 to 20 m/min [Fig. 2 and Breen et al. Fig. 5 (7)]. However, we did not observe a further increase in VEGF mRNA when the treadmill speed was increased to 25 m/min (Fig. 2). One possible explanation for these results would suggest that oxidative fibers are incapable of producing VEGF, and thus the recruitment of these fibers as treadmill speed increases does not result in increased VEGF gene expression. A second possibility is that all fiber types may be capable of producing VEGF but that VEGF production is maximal during moderate exercise intensities. A third possibility is that, as more fibers are recruited at higher treadmill speeds, the more oxidative fibers may selectively produce less VEGF, whereas the less oxidative fibers may produce more VEGF, thus resulting in no increase in total VEGF mRNA within the gastrocnemius. Further work is clearly required to determine the relationship between exercise-induced VEGF gene expression and fiber type.

Growth factor gene expression regulation in response to acute exercise. One theory regarding EIA in skeletal muscle suggests that prolonged imbalances between the perfusion capabilities of blood vessels and the metabolic requirements of tissue cells leads to decreased oxygenation, with the long-term result being a modification of the vasculature to satisfy the tissue needs (1). In response to greater work intensities and the greater demand for ATP production, blood flow is increased to promote the delivery of substrates, including O2, and the removal of metabolites. Increased blood flow is the principal defense for maintaining capillary PO2 and therefore the driving force for O2 consumption (22). It has been hypothesized that intracellular PO2 falls progressively in response to increases in work (35). However, experimental data in humans do not support this hypothesis (30). In humans, intracellular PO2 falls rapidly on commencement of exercise and progressively during exercise at intensities less than ~50% of VO2 max. Further increases in work rate, however, do not further reduce intracellular PO2. Our results from
protocol A are consistent with the hypothesis that intracellular P O2 can regulate skeletal muscle VEGF and TGF-β1 gene expression in that we show no greater increase in VEGF or TGF-β1 mRNA when work rate was increased above 50%, a workload during which intracellular P O2 would not be expected to change on the basis of human measurements of intracellular P O2. Whether the same relationship of intracellular P O2 to exercise intensity holds for the rat is unknown.

Richardson et al. (31) investigated the effect of acute normoxic and hypoxic exercise on the exercise-induced increase in VEGF mRNA in humans. Despite a significant reduction in intracellular P O2 during hypoxic exercise (7.2 vs. 3.8 Torr), the exercise-induced increase in VEGF mRNA was similar between normoxic and hypoxic exercise, although paradoxically less in response to hypoxic exercise. The authors suggest that, as P O2 falls from rest to exercise, this drop may achieve an intracellular P O2 threshold that would stimulate VEGF. In relative contrast, Gustafsson et al. (17) found no significant increase but a trend toward a greater exercise-induced VEGF mRNA response during reduced leg blood flow that lowered venous P O2 during exercise (22.3 vs. 19.3 Torr). In the study by Richardson et al. (31), the workload was ~50% of maximum, whereas in the report from Gustafsson et al. (17) it was 24% of maximum. In the original work by Breen et al. (7) in the rat, the workload was ~40% of maximum. Perhaps the discrepancies among these studies may be explained by our results, in which increases in treadmill speed above 50% do not produce any further increase in VEGF mRNA. As suggested by Richardson et al. (31), there may be a threshold P O2 below which further reductions in intracellular P O2 do not produce further increases in the exercise-induced VEGF mRNA response.

It has been suggested that mechanical events produced by the increased blood flow associated with exercise are responsible for exercise-induced angiogenesis (23). In this theory, increased blood flow would increase 1) interaction of blood components with endothelial cells; 2) shear stress; and 3) wall tension. In rats, increases in treadmill speed promote increases in muscle blood flow (27). If angiogenic growth factor gene expression were regulated by increases in blood flow during systemic exercise, we would expect that growth factor gene expression would be increased further by increases in workload, which we did not observe (Figs. 1 and 2). This is in agreement with Roca et al. (33), who demonstrated in isolated canine hindlimb that passive hyperperfusion does not increase VEGF or bFGF mRNA, whereas TGF-β1 mRNA was slightly but significantly increased. It might be hypothesized that passive hyperperfusion, as performed by Roca et al., would increase the release of vasodilators, including the release of nitric oxide (NO), in response to the increase in shear stress (25). NO has been shown to regulate VEGF gene expression both in resting and exercising skeletal muscle (5, 11). Thus, if NO was released during passive hyperperfusion, it did not increase VEGF mRNA. Recently, we demonstrated that NO synthase (NOS) inhibition via nitro-L-arginine methyl ester (L-NAME) attenuates the exercise-induced increase in VEGF mRNA (11). In this report, we also demonstrated that D-NAME, the inactive enantiomer of L-NAME, increased mean arterial pressure to the same extent as a similar dosage of L-NAME, suggesting that some NOS inhibition had occurred. However, D-NAME did not affect the exercise-induced increase in VEGF gene expression (11). This result suggests that either different NOS isoforms or the location of different NOS isoforms within the vasculature and muscle fibers may differentially and selectively regulate blood pressure and VEGF gene expression.

Growth factor gene expression regulation in response to short-term exercise training. In addition to tissue hypoxia, various other metabolites have been impli-
cated as regulators of angiogenesis during exercise. Among these are increases in lactate, reductions in pH, inadequate rephosphorylation of ATP, and subsequent accumulation of ADP and adenosine (23). With an increase in oxidative potential, less perturbation is observed in many oxidative metabolites (6). Less perturbation in PCr, Cr, and Pi during submaximal exercise can be observed after just 3–4 days of endurance exercise training (13). There are reports that CSA, a commonly measured enzymatic marker of oxidative potential, can increase within the first week of an exercise training program (9, 28, 37, 39). Recently, Richardson et al. (32) showed that, after 8 wk of exercise training, which produced increases in CSA, aerobic capacity, and capillarization, VEGF mRNA was reduced in response to acute exercise. Because exercise training increases oxidative potential, with the accompanying improvement in cell homeostasis being consistent with the metabolic theory of angiogenesis, we had hypothesized that possible reductions in growth factor gene expression during the 5 days of exercise training would be related to increases in oxidative potential. Contrary to this hypothesis, the smaller exercise-induced increase in VEGF mRNA observed after exercise on days 2–5 was not coincident with an increase in CSA (Figs. 4 and 5). Although increased oxidative potential does not explain the reduction in the exercise-induced increase in VEGF mRNA on days 2–5, it must be noted that both VEGF and TGF-β1 mRNA are increased throughout the 5 days of exercise training. Our results do not preclude the hypothesis that the regulators of exercise-induced growth factor gene expression may originate from metabolism.

The finding that CSA was unchanged in response to our exercise training protocol is not unique. Short-term exercise training does not always increase CSA (14, 15). The protocol used in this report was designed to produce the greatest exercise-induced increase in angiogenic growth factor responses (Figs. 1 and 2) but may not have been of sufficient intensity and duration to promote increases in CSA within 5 days. However, other reports using similar training regimes, but last-
In monocytic and endothelial cells, hypoglycemia increases VEGF expression (34, 40). If glucose regulates VEGF gene expression, an improvement in muscle glucose availability would reduce a potential stimulus for VEGF gene upregulation and could explain the smaller increase we observed in VEGF gene expression after exercise on days 2–5. Short-term exercise training increases exercise duration to fatigue. Muscle glycogen is an essential fuel for prolonged aerobic exercise, and glycogen availability would therefore be greater for subsequent exercise bouts than for the initial bout. In untrained individuals, 3 days of consecutive exercise training substantially elevates submaximal exercise tolerance with the increase in exercise intensity requiring increased reliance on muscle glycogen (24). Exercise produces a significant reduction in muscle glycogen (14, 16, 24). An essential component of recovery from prolonged exercise is the replenishment of muscle glycogen. Within 5 h after the completion of a single exercise bout in untrained rats, not only can muscle glycogen levels be restored to preexercise levels, but glycogen stores can be significantly elevated above preexercise levels (24). Glycogen availability would therefore be greater for subsequent exercise bouts than for the initial bout. In untrained individuals, 3 days of consecutive exercise training substantially elevates submaximal exercise tolerance with the increase in resistance to fatigue being related to elevated glycogen availability (12). Thus the smaller exercise-induced increase in VEGF mRNA on days 2–5 could result from expected increases in glucose availability after the initial exercise bout.

In animal models, chronic electrical nerve stimulation (CENS) has been used to investigate long-term changes in VEGF gene expression. Similar to our model, CENS increases VEGF mRNA (20, 36). It must be noted that the characteristics of CENS-induced angiogenesis can differ considerably compared with traditional exercise. Traditional exercise increases skeletal muscle capillarization 10–30%, requiring weeks to accomplish this increase (2, 18, 19, 23, 26, 41). In contrast, CENS can often lead to twofold increases in capillarization that can be observed within 1–2 days after the initiation of CENS (36). In general, CENS produces an initial increase in VEGF mRNA followed by a progressive decrease over time (20, 36). The relationship between VEGF expression and capillary growth with CENS supports the hypothesis that VEGF expression is controlled by a negative feedback mechanism, with tissue hypoxia being the regulated variable (20). As mentioned above, Richardson et al. (32) recently reported an increase in muscle capillarization along with a concomitant reduction in the exercise-induced increase in VEGF mRNA after 8 wk of exercise training.

In the present report, we were unable to demonstrate a measurable increase in bFGF mRNA in protocol B. This is consistent with previous reports in which increases in VEGF and TGF-$\beta_1$ mRNA, but not bFGF mRNA, were demonstrated after 1 h of exercise at 20 m/min, 10° incline (10, 11), but in contrast to Breen et al. (7) and our results from protocol A. In the present report, every effort was made to remove all muscle samples in an expedient fashion. We have hypothesized previously that our inability to demonstrate an increase in bFGF mRNA when muscle samples were harvested ~40 min after exercise was due to the rapid decline in the exercise-induced increase in bFGF gene expression (10). Breen et al. (7) demonstrated that bFGF mRNA was increased only immediately after exercise, whereas both VEGF and TGF-$\beta_1$ were still elevated 2 h after exercise. In the present report, the observation that bFGF mRNA is not consistently increased with exercise may suggest an unintentional and random yet systematic bias to a small difference in the time required for tissue removal between the two protocols.

In summary, we have demonstrated that the angiogenic growth factor responses to a single bout of exercise in the rat are not increased when exercise intensity is increased above 50% of maximum. This relationship is consistent with the hypothesis that intracellular PO$_2$ regulates VEGF and TGF-$\beta_1$ gene expression. During the initial stages of an exercise training program, both VEGF and TGF-$\beta_1$ mRNA are consistently increased in response to exercise. However, a smaller increase in VEGF mRNA was observed after exercise on days 2–5 (compared with day 1). The lower VEGF mRNA on days 2–5 is not related to increases in oxidative potential.

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