Chronic hypoxia attenuates resting and exercise-induced VEGF, flt-1, and flk-1 mRNA levels in skeletal muscle

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Olfert, I. Mark, Ellen C. Breen, Odile Mathieu-Costello, and Peter D. Wagner. Chronic hypoxia attenuates resting and exercise-induced VEGF, flt-1, and flk-1 mRNA levels in skeletal muscle. J Appl Physiol 90: 1532–1538, 2001.—Vascular endothelial growth factor (VEGF) is a hypoxia-inducible angiogenic mitogen. However, chronic hypoxia is generally not found to increase mammalian skeletal muscle capillarity. We sought to determine the effect of chronic hypoxia (8 wk, inspired O2 fraction = 0.12) on skeletal muscle gene expression of VEGF, its receptors (flt-1 and flk-1), basic fibroblast growth factor, and transforming growth factor-β. Wistar rats were exposed to chronic hypoxia (n = 12) or room air (n = 12). After the exposure period, six animals from each group were subjected to a single 1-h treadmill exercise bout (18 m/min on a 10° incline) in room air while the remaining six animals served as rest controls. Morphological analysis revealed that chronic hypoxia did not increase skeletal muscle capillarity. Northern blot analyses showed that chronic hypoxia decreased resting VEGF, flt-1, and flk-1 mRNA by 23, 68, and 42%, respectively (P < 0.05). The VEGF mRNA response to exercise was also decreased (4.1- and 2.7-fold increase in room air and chronic hypoxia, respectively, P < 0.05). In contrast, neither transforming growth factor-β, nor basic fibroblast growth factor mRNA was significantly altered by chronic hypoxia. In conclusion, prolonged exposure to hypoxia attenuated gene expression of VEGF and its receptors flt-1 and flk-1 in rat gastrocnemius muscle. These findings may provide an explanation for the lack of mammalian skeletal muscle angiogenesis that is observed after chronic hypoxia.

Northern blot analysis; angiogenesis; transforming growth factor-β1; basic fibroblast growth factor

THE EFFECTS OF CHRONIC HYPOXIA have been well documented in humans and animals (1). Initial studies in skeletal muscle reported an increase in capillary density in response to chronic hypoxia. However, later studies revealed that the observed increase in capillary density was not due to new capillary growth but, rather, a reduction in muscle fiber area (attributed to muscle atrophy or tissue shrinkage associated with the histological technique) (1, 39). Similarly, in other cases where increases in muscle capillarity were observed, factors such as the cold temperature (associated with high altitude) (2) and normal growth and development (34) were found to contribute to capillary growth. Therefore, it has widely been accepted that chronic hypoxia alone does not induce skeletal muscle angiogenesis. Indeed, Banchero (1), in 1987, after reviewing the bulk of existing evidence, concluded that “skeletal muscle capillarity does not respond to simple normothermic hypoxia.” Nevertheless, more recently it has been reported that chronic hypoxia increases skeletal muscle capillarity (as measured by capillary-to-fiber ratio) in several avian species (20, 23, 28) and possibly in the rat (10). Moreover, chronic hypoxia is also found to increase the number of capillaries in the mammalian brain (4) and placenta (37). Therefore, whether chronic hypoxia is capable of inducing angiogenesis remains controversial.

Within the last decade, vascular endothelial growth factor (VEGF) has emerged as an important hypoxia-inducible angiogenic mitogen (13). VEGF is expressed by a wide variety of cell types and is known to increase vascular permeability, endothelial cell proliferation, migration, and angiogenesis in vivo (12). The VEGF receptors flt-1 and flk-1 are found almost exclusively on vascular endothelial cells, making VEGF an endothelium-specific mitogen (12). There is little question regarding the importance of VEGF in the initiation of angiogenesis, and its essential role is emphasized by the fact that gene inactivation of VEGF or its receptors results in an early embryonic death (12).

Hypoxia has been found to be an important stimulus that induces transcriptional induction of VEGF and increases posttranscriptional stabilization of VEGF mRNA (9, 25). In addition to hypoxia, several cytokines (interleukin-1β, -10, and -13), growth factors [basic fibroblast growth factor (bFGF) and transforming growth factor-β1 (TGF-β1)], and vasoactive molecules [nitric oxide (NO) and adenosine] have been shown to regulate VEGF gene expression (3, 12, 30). Several studies have now shown that VEGF mRNA is increased in skeletal muscle after acute exercise and that...
acute hypoxic exercise further augments the VEGF mRNA response (5, 18, 32). Similarly, electrical nerve stimulation, which is frequently used to simulate exercise, is also found to increase VEGF mRNA levels (19). Indeed, exercise training and electrical stimulation are well known to increase skeletal muscle capillarity, indicating that VEGF is likely to play an important role in skeletal muscle angiogenesis.

Because VEGF is believed to be the primary mediator in hypoxia-induced angiogenesis, it seems paradoxical that increases in capillarity are not routinely found in mammalian skeletal muscle exposed to chronic hypoxia. Therefore, we sought to determine what effect chronic hypoxia might have on the gene expression of VEGF and its receptors (flt-1 and flk-1). Gene expression of two other growth factors (bFGF and TGF-β1), both of which demonstrate angiogenic activity and may also regulate VEGF, was also studied.

In this study we demonstrate, using quantitative Northern blot analysis, that rats exposed to chronic hypoxia, at a level that increases muscle VEGF mRNA acutely (5), decreased the resting mRNA levels of VEGF, flt-1, and flk-1 in the gastrocnemius muscle. In addition, chronic hypoxia did not increase the capillary-to-fiber ratio or the number of capillaries around a fiber in this muscle. Chronic hypoxia also attenuated the VEGF mRNA response to exercise. In contrast, neither resting nor exercise-induced levels of bFGF and TGF-β1 mRNAs were significantly altered by chronic hypoxia.

MATERIALS AND METHODS

This study was approved by the University of California, San Diego, Animal Subjects Committee and the Loma Linda University Animal Research Committee. Twenty-four 9- to 10-wk-old female Wistar rats were randomly assigned to room air (n = 12) or chronic hypoxia (n = 12, inspired O₂ fraction (FIO₂) = 0.12) for 8 wk. This FIO₂ was based on prior work showing acute increases in VEGF mRNA in skeletal muscle of rats exposed to this level of O₂ for just 1 h (5). After 8 wk at the assigned FIO₂, six animals from each group were subjected to a single 1-h exercise bout while the remaining six animals served as resting (i.e., basal) controls. This strategy allowed us to examine the effect of chronic hypoxia on the basal, as well as exercised-induced, gene expression levels in skeletal muscle.

Environmental conditions. Normobaric hypoxia was maintained using an environmental chamber that electronically controlled the FIO₂ level by mixing air and N₂ from tanks. Separate O₂ and CO₂ analyzers independently monitored and continuously recorded O₂ and CO₂ levels throughout the 8-wk exposure period. FIO₂ was maintained at 0.12, and CO₂ levels were kept below 0.5% by using soda lime. The environmental chamber also electronically controlled and maintained normal ambient temperature (22–24°C) within the chamber. All animals were visually inspected daily for abnormal and normal behavior, food and water consumption, and overall health. Cages were cleaned and bedding was replaced every 2–3 days or as needed between the scheduled changes. Animals were provided standard rat food and water ad libitum and were confined to their cages (2–3/cage) throughout the 8-wk period.

Single exercise bout. After the 8-wk exposure period, six animals from each group were randomly selected to perform the single exercise bout. The rats were allowed to familiarize themselves with the rodent treadmill (Omnipacer model CL-4, Omnitech, Columbus, OH) for 5–10 min at a slow speed (5 m/min), and then they ran for 1 h at 18 m/min on a 10° incline in room air. To motivate the rats and keep them running, a shock grid and air jets were utilized at the rear of the treadmill.

Surgical procedure and vascular perfusion protocol. After they completed the exercise bout, the rats were immediately anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg ip). A corneal reflex or toe pinch response was used to ensure adequate anesthesia; then the entire left gastrocnemius muscle was surgically removed and flash frozen using liquid nitrogen. Muscles were stored at −80°C until processed for Northern blot analysis.

The remaining gastrocnemius muscle (right side) was perfused with a 6.25% solution of glutaraldehyde in a 0.1 M sodium cacodylate buffer (total osmolarity of the fixative 106 g/liter, pH 7.4) for 10 min using a whole body perfusion technique described by Mathieu-Costello (27). The gastrocnemius muscle was then carefully excised and stored at 4°C in a glutaraldehyde solution until processed for morphometric analysis. With the use of anatomic landmarks, a small portion of muscle was taken from the deep midbulb portion of the medial gastrocnemius muscle, from an area of mixed fiber type distribution that has been shown to consist of slow-twitch oxidative (~12%), fast-twitch glycolytic (~46%), and fast-twitch oxidative-glycolytic (~42%) fiber types (22). All samples were cut (minced) into small longitudinal strips, dehydrated, and embedded in resin blocks. From each animal, four blocks were cut into four (1-μm-thick) transverse sections using a LKB Ultrrotome III and stained with 0.1% aqueous toluidine blue solution, as previously described (27). With the use of a light microscope, fiber cross-sectional area, capillary density (capillary number/fiber cross-sectional area), and the number of capillaries around a fiber were measured. The ratio of the number of capillaries to the number of fibers was calculated by multiplying capillary density by fiber cross-sectional area.

Animals that were not exercised (i.e., rest) underwent the same surgical and vascular perfusion procedure.

RNA isolation and Northern blot analysis. Total cellular RNA was isolated from the whole left medial gastrocnemius muscle by the method of Chomczynski and Sacchi (7). RNA (10 μg) preparations were quantitated by absorbance at 260 nm, and intactness was assessed by ethidium bromide staining of RNA that was separated by electrophoresis using a 6.6% formaldehyde-1% agarose gel. Fractionated RNA was transferred to a Zeta probe membrane (Bio-Rad, Hercules, CA), cross-linked to the nylon membrane by ultraviolet irradiation, and then stored at 4°C. Blots were then probed with oligolabeled [α-32P]dCTP cDNA probes, which had specific activities of ≥1 × 108 disintegrations·min⁻¹·μg DNA⁻¹ (11).

The rat VEGF probe is a 0.9-kb cDNA Pet/Smal insert cloned into pBluescript II KS(+) vector. The 1.2-kb rat KDR/Flik-1 cDNA EcoRI insert of pUC18 and the 0.6-kb rat Flt-1 cDNA EcoRI/HindIII insert of pUC119 were kindly provided by Dr. Masabumi Shibuya (42). The rat TGF-β1 cDNA probe is a 0.8-kb cDNA Pet/Smal insert cloned into plBluescript II KS(+) vector. The bFGF probe is a 1-kb XhoI fragment of human bFGF cDNA. Prehybridization and hybridizations were performed in 50% formamide, 10× saline-sodium citrate (SSC, 20× SSC is 0.3 M sodium chloride and 0.3 M sodium citrate), 5× Denhardt’s solution (100× Denhardt’s solution is 2% Ficoll and 2% polyvinylpyrrolidone), 50 mM

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sodium phosphate (pH 6.5), 1% SDS, and 250 μg/ml sonicated 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 65°C for VEGF mRNA, 60°C for bFGF mRNA, and 50°C for TGF-β1, Flk-1, and Flt-1 mRNAs. Blots were exposed to X-Omat AR-5 X-ray film (Eastman Kodak, New Haven, CT) using a Cronex Lighting Plus screen at -80°C. Autoradiographs were quantitated via a computer densitometry software package (Gel-Pro Analyzer, Media Cybernetics, Silver Spring, MD) and normalized to 18S rRNA levels.

Data analysis. Values are means ± SE. ANOVA and Student’s t-test were used to determine significance. Significance was accepted at the 0.05 confidence level.

RESULTS

As expected, rats exposed to chronic hypoxia weighed significantly less than rats exposed to room air (230 ± 3.7 vs. 243 ± 4.2 g, P < 0.05; Fig. 1). Chronic hypoxia did not increase skeletal muscle capillarity in the rat gastrocnemius muscle (Fig. 2). This is also evident in Table 1, where morphological analyses revealed no significant difference in skeletal muscle capillarity (i.e., number of capillaries around a fiber and capillary-to-fiber ratio).

Northern blot analyses of the effect of chronic hypoxia on resting and exercise-induced VEGF, TGF-β1, bFGF, flt-1, and flk-1 mRNA levels are shown in Fig. 3. Densitometric analyses, compared with room air resting values, which were normalized to 1.0 (Fig. 4), revealed that chronic hypoxia lowered resting levels of VEGF mRNA (1.0 ± 0.03 and 0.77 ± 0.08 in room air and chronic hypoxia, respectively, P < 0.05), whereas bFGF (1.0 ± 0.05 and 1.11 ± 0.04 in room air and chronic hypoxia, respectively, not significant) and TGF-β1 (1.0 ± 0.10 and 0.68 ± 0.06 in room air and chronic hypoxia, respectively, not significant) mRNA levels were not significantly altered.

We also confirmed that VEGF mRNA increased four-fold from rest to exercise (Fig. 4) in room air-breathing rats (1.0 ± 0.03 and 4.09 ± 0.69 at rest and after a single exercise bout, respectively, P < 0.05), as previously reported by Breen et al. (5). However, we found that chronic hypoxia attenuated this response (4.1- and 2.7-fold in room air and chronic hypoxia, respectively, P < 0.05), suggesting that the VEGF gene response to exercise has been downregulated. Although neither TGF-β1 (P = 0.06) nor bFGF mRNA (P = 0.19) increased significantly in room air-breathing animals after exercise, a small but statistically significant increase in bFGF mRNA was found after exercise in animals exposed to chronic hypoxia compared with those exposed to room air (Fig. 4).

Chronic hypoxia also decreased resting levels of flt-1 and flk-1 mRNA (Fig. 5). Interestingly, flt-1 and flk-1 mRNA exhibited divergent responses to exercise. Flk-1 mRNA decreased with exercise in animals exposed to room air and chronic hypoxia, but this decrease was statistically significant only in the animals exposed to room air (1.0 ± 0.10 and 0.47 ± 0.05 at rest and after a single exercise bout, respectively, P < 0.05). In contrast, flt-1 mRNA tended to increase with exercise, but this was not statistically significant in the animals exposed to room air or chronic hypoxia.

DISCUSSION

The principal finding in this study is that chronic hypoxia decreases the gene expression of VEGF and its
receptors flt-1 and flk-1 in mammalian skeletal muscle. Exposure to chronic hypoxia also attenuated, but did not abolish, the VEGF mRNA response to a single bout of exercise. These findings suggest that chronic hypoxia alters the gene expression of VEGF and its receptors in a manner that is unfavorable to capillary growth and may explain why an increase in mammalian skeletal muscle angiogenesis is not generally observed after chronic hypoxia.

Chronic hypoxia and muscle morphology. In most studies, chronic hypoxia or high-altitude exposure caused a reduction in muscle fiber area (usually attributed to muscle atrophy). On morphological analysis (Table 1), we did not find a decrease in the muscle fiber area (fiber cross-sectional area), nor did we find a concomitant increase in capillary density. Although, at first, the lack of decrease in fiber size may seem inconsistent, others who have exposed rats to a similar level of hypoxia (FiO₂ = 0.12 or ~4,000-m altitude) have also not found significant decreases in fiber cross-sectional area (40). Indeed, most studies (6) that have found a significant decrease in fiber cross-sectional area were conducted at altitudes >5,000 m (equivalent to FiO₂ ≤ 0.10). Therefore, it is not surprising (given the level of hypoxia) that we did not find a reduction in fiber cross-sectional area. However, consistent with most studies in mammals, we found that chronic hypoxia did not increase the number of capillaries around a fiber or the capillary-to-fiber ratio, indicating that new capillaries had not been formed. One explanation for the lack of capillary change could be that, to the extent intracellular hypoxia is the stimulus for VEGF gene expression, intracellular Po₂ was, in fact, not sufficiently reduced by chronic hypoxia to provide an adequate stimulus. However, our laboratory’s prior work showing that acute 1-h exposure of similar rats to the same FiO₂ used here (0.12) did, in fact, produce a significant (>2-fold) increase in VEGF mRNA (5) would argue against this possibility.

Perhaps the most observable physical change with chronic hypoxia exposure is a reduction in body weight. Body weight was significantly lower in animals ex-

![Fig. 3. Quantitative Northern blots showing the effect of chronic hypoxia on vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-β1 (TGF-β1), flt-1, and flk-1 mRNA in rat gastrocnemius muscle under steady state (rest) and immediately after exercise; 10 μg of total RNA were loaded in each lane. 18S rRNA demonstrates equal loading of total RNA per lane.](image)

![Fig. 4. Angiogenic growth factor mRNA response in gastrocnemius muscle after a single exercise bout in rats maintained in room air or chronic hypoxia (12% O₂). Data reflect the magnitude change compared with the room air resting value, which was normalized to 1.0. *Significant response with exercise, P < 0.05; **significant change compared with room air, P < 0.05; ns, not significant.](image)
posed to chronic hypoxia than in room air controls (Fig. 1). Because metabolic demands are generally thought to regulate the vascularization of tissues and tumors, it is possible that factors such as loss of appetite or decreased level of activity may play a role in regulating capillary growth and, therefore, may also regulate VEGF gene expression. However, on daily visual inspection, there was no noticeable difference in food consumption. Chronically hypoxic rats appeared to sleep more than normoxic rats and, therefore, may have been less active. Because decreasing muscle activity (e.g., cutting a tendon or immobilizing a limb in a plaster cast) results in muscle atrophy and can dramatically decrease muscle vascularity, it is possible that a reduction in activity time may have played a role in attenuating VEGF mRNA levels. However, cage activity and behavior while the animals are awake (e.g., grooming and moving around the cage) were not noticeably different between groups, and perhaps more importantly, neither muscle atrophy (as measured from muscle fiber cross-sectional area) nor a reduction in muscle vascularity was observed in rats subjected to chronic hypoxia (Table 1, Fig. 2). Therefore, it seems unlikely that the difference in cage activity time and, therefore, level of activity, if any, would account for the changes in VEGF gene expression we observed, especially considering the findings that basal (i.e., resting) levels of VEGF mRNA in the skeletal muscle of humans (33) and rats (Olfert, unpublished observations) have been found not to be significantly different between less active untrained muscles and those that have been exercise trained.

**Chronic hypoxia and VEGF gene expression.** It is well known that hypoxia induces VEGF gene expression. This increase is now known to be mediated through an O2-responsive transcriptional factor, hypoxia inducible factor-1 (HIF-1) (25, 38). Although some studies (8, 41), mostly in the lung, have reported that chronic hypoxia increases VEGF gene expression, a recent study in the mouse brain showed that VEGF protein and gene expression return to basal levels after 7 days of continuous hypoxia (21). This finding is consistent with our data in the rat gastrocnemius muscle (Figs. 3 and 4) and suggests that a mechanism exists by which chronic hypoxia can downregulate VEGF gene expression. In support of this observation, Levy (24) recently demonstrated that transcription of VEGF (through HIF-1) is inhibited or severely blunted in human hepatoma (Hep 3B) cells and rat cardiomyocytes (H9c2) preconditioned to a hypoxic environment (i.e., grown in culture under hypoxia). Similarly, cultured neurons preconditioned to hypoxia also demonstrate decreased HIF-1 binding activity (35). Moreover, in cultured aortic smooth muscle cells, NO, which is believed to be important in the vasodilatory response to hypoxia, has also been shown to inhibit hypoxic induction of VEGF by decreased HIF-1 binding activity (26). Therefore, although acute hypoxia has been found to induce VEGF gene expression, prolonged exposure to hypoxia (i.e., weeks) appears to eventually downregulate transcriptional activation of VEGF in a variety of cells and tissues.

However, recent studies by Benoit et al. (3) and Gavin et al. (15) have demonstrated that NO is important in the VEGF gene response to exercise in the rat gastrocnemius. Therefore, whether NO, after chronic hypoxic exposure, is truly involved in attenuating skeletal muscle VEGF gene expression is unclear. Additionally, whether skeletal muscle VEGF protein levels are also decreased after chronic hypoxia is also unknown. Although it is true that changes in mRNA do not necessarily translate to changes at the protein level, given the importance of VEGF in angiogenesis, it would be surprising to find a relationship between VEGF mRNA and angiogenesis without a resultant change at the protein level.

**Exercise-induced gene expression and chronic hypoxia.** Exercise induces a number of changes within the cardiovascular and skeletal muscle systems, such as increased blood flow, changes in local metabolite concentrations, increased mechanical stresses, and acid-base changes. However, the mechanism by which genes are induced or regulated in response to exercise
remains poorly understood. It is well known that exercise produces a substantial drop in extracellular (venous) and, perhaps more importantly, intracellular skeletal muscle PO$_2$ (31). Therefore, it is possible that a local reduction in PO$_2$ may regulate VEGF transcription in response to exercise. Accordingly, if hypoxic induction of VEGF is attenuated by chronic hypoxia due to decreased HIF-1 binding activity, exercise-induced gene expression may also be attenuated. Indeed, the VEGF gene response to exercise was attenuated by chronic hypoxia (Fig. 4). This is consistent with the notion that a local hypoxia might be acting as a stimulus that induces VEGF gene expression.

VEGF receptors (Flt-1 and Flk-1) and exercise. Both flt-1 and flk-1 receptors have been shown to be important in angiogenesis (12). Therefore, our finding that chronic hypoxia attenuates resting levels of flt-1 and flk-1 mRNA is consistent with the notion that chronic hypoxia alters skeletal muscle in a manner that is unlikely to favor capillary growth. Perhaps of greater interest is the finding that the flt-1 and flk-1 responded divergently to exercise, which is consistent with the observation that hypoxia at the transcriptional level appears to differentially regulate these VEGF receptors. Indeed, Sandner et al. (36) reported that hypoxia increases flt-1 mRNA, but not flk-1 mRNA, in the rat liver and lungs, and Gerber et al. (17) demonstrated that gene expression of flt-1, but not flk-1, is regulated through HIF-1. In our study, flt-1 mRNA levels tended to increase with exercise, which supports the notion of hypoxia at the tissue level may play a role in regulating gene transcription in response to exercise. Indeed, other studies have found flt-1 mRNA to significantly increase with exercise (14, 16). The fact that flt-1 mRNA was not increased significantly in our animals may be explained by the time at which muscles were sampled. In contrast to VEGF, in which peak gene expression occurs rapidly (within 0–2 h) in response to exercise (5), the peak VEGF receptor gene response to exercise may not occur until several hours after exercise (16). In this study, muscles were removed immediately (<20 min) after exercise, whereas the muscles in the studies that demonstrated a significant rise in flt-1 mRNA were removed ≥1 h after exercise (allowing greater time for mRNA levels to increase after exercise). Given the importance of VEGF and its receptors in angiogenesis, it seems reasonable to believe that at least one VEGF receptor (e.g., flt-1), if not both, is involved in exercise-induced angiogenesis.

TGF-$\beta_1$ and bFGF gene expression. Breen et al. (5) showed a small, but significant, increase in TGF-$\beta_1$ and bFGF mRNA with exercise in the rat gastrocnemius. Although we do not report a significant increase in TGF-$\beta_1$ ($P = 0.06$) or bFGF in our experimental animals, it is evident in Fig. 4 that TGF-$\beta_1$ mRNA levels tended to increase with exercise, and $P = 0.06$ is on the borderline of the standard level of significance. These findings are consistent with those of others (15) demonstrating only a modest increase in TGF-$\beta_1$ mRNA and no changes in bFGF mRNA in response to exercise. Given the relatively small gene responses in TGF-$\beta_1$ and bFGF in all three studies, it seems reasonable to believe that neither growth factor is likely to play a significant role in the acute angiogenic response to exercise. Nonetheless, it is important to note that both growth factors have been shown to be important angiogenic regulators. Moreover, chronic electrical nerve stimulation (>3 wk) has been shown to increase bFGF protein in skeletal muscle (29), suggesting that bFGF may be involved in the angiogenic response to repeated exercise bouts. However, the present results suggest that it is unlikely that TGF-$\beta_1$ or bFGF plays a significant role in regulation of skeletal muscle capillarity in chronic hypoxia.

In summary, 8 wk of chronic hypoxia ($F_{\text{IO}_2} = 0.12$) was found not to increase the number capillaries in rat gastrocnemius muscle. Moreover, gene expression of VEGF and its receptors was attenuated by chronic hypoxia in the rat gastrocnemius. The VEGF mRNA response to exercise was also attenuated by chronic hypoxia. Although the mechanism by which chronic hypoxia regulates the gene expression of VEGF and its receptors remains to be identified, these findings may explain, at least partially, why increases in skeletal muscle capillarity are not routinely found in mammals exposed to chronic hypoxia or high altitude.

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