Muscle angiogenic growth factor gene responses to exercise in chronic renal failure

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Wagner, Peter D., Ferran Masanés, Harrieth Wagner, Ernest Sala, Oscar Miró, J. M. Campistol, Ramon M. Marrades, Jordi Casademont, V. Torregrosa, and Josep Roca. Muscle angiogenic growth factor gene responses to exercise in chronic renal failure. Am J Physiol Regulatory Integrative Comp Physiol 281: R539–R546, 2001.—Patients with chronic renal failure (CRF) have impaired exercise capacity even after erythropoietin treatment. We recently showed that although this is explained in part by reduced convective O2 delivery to muscles, there is also an impairment of O2 transport from muscle capillaries to the mitochondria. Given the importance of the capillary surface area for capillary mitochondrial O2 transport and reports of reduced capillarity in CRF, we hypothesized that the angiogenic gene response to exercise is impaired in such patients. Six patients with CRF and six control subjects matched for age, size, and sedentary lifestyle exercised on a single occasion for 1 h at similar work intensities averaging 50% of maximal capacity. Exercise was confined to the knee extensors of a single leg by means of a specially designed leg-kick ergometer. A percutaneous biopsy of the quadriceps was taken within 30 min of cessation of exercise and compared with a similar biopsy done at different times without any prior exercise for 24 h. Conventional Northern blots were prepared and probed for vascular endothelial growth factor (VEGF; the major putative angiogenic growth factor for muscle), basic fibroblast growth factor (bFGF), and transforming growth factor (TGF)-β1. Data during both rest and exercise were successfully obtained in four subjects of each group. We also assessed muscle capillarity and mitochondrial oxidative capacity to relate to these changes. Mitochondrial oxidative capacity was normal, whereas capillary number per fiber was 12% lower than in normal subjects. VEGF mRNA abundance was increased after exercise by about one order of magnitude, with no reduction in response in CRF. For bFGF and TGF-β1, exercise elicited no response in either group. Reduced muscle capillarity in CRF does not, therefore, stem from reduced transcription of VEGF. To the extent that VEGF is important to exercise-induced angiogenesis in muscle, we suspect a posttranscriptional aberration in this response occurs in CRF to explain reduced capillarity.

PATIENTS WITH CHRONIC RENAL failure are well known to have reduced exercise capacity (20, 24, 28), which fails to normalize after the usually severe anemia of this condition has been substantially reversed by erythropoietin (19, 20, 25). This observation has led us to hypothesize that intrinsic muscle abnormalities may contribute to exercise limitation in such patients. This could be via O2 transport defects or metabolic insufficiency.

An important component of the O2 transport pathway that may contribute to limiting exercise capacity is the richness of the muscle microvascular network. We have shown that the amount of capillary surface (rather than the distance O2 must diffuse from red blood cells to mitochondria) is a key factor in the overall conductance of O2 to the cytochromes (12). Mathieu-Costello et al. (21–23) have shown that, across species, capillary surface is clearly related to mitochondrial density. Others have shown that although muscle intravascular PO2 is high (20–100 Torr), average intracellular PO2 in muscle is one order of magnitude lower (13), a finding confirmed in humans by [1H]myoglobin magnetic resonance spectroscopy (MRS) (31). When these observations were considered along with reports of reduced muscle O2 conductance (20) and reduced muscle capillarity in renal failure (14, 28), we were led to the hypothesis that in chronic renal failure, exercise capacity is reduced in part by reduced muscle O2 conductance that in turn is caused by subnormal amounts of capillary surface area for O2 diffusion from red blood cell to muscle cell. A potential explanation for this is, therefore, impaired angiogenesis.

Accordingly, in a group of six young patients with chronic renal failure and a group of six age-, size-, and...
activity-matched normal control subjects, we examined from biopsy samples the angiogenic growth factor response to a single, prolonged bout of exercise. Specifically, we expected to see a reduced effect of exercise on previously described increases in angiogenic growth factor mRNA (2, 11). Our focus was vascular endothelial growth factor (VEGF), but, based on prior work in the rat (2), we also examined mRNA responses of two other growth factors involved in angiogenesis: basic fibroblast growth factor (bFGF) and transforming growth factor-β3 (TGF-β3). We related these findings to structural and biochemical data from the same biopsy material.

METHODS

Subjects. Six patients with chronic renal failure, already treated with erythropoietin to overcome their anemia, agreed to this study, which had been approved by University of Barcelona Human Subjects Committee. Written informed consent was obtained. The clinical details of these subjects appear elsewhere (35). Mean age, weight, and height were 25 ± 6 yr, 67 ± 10 kg, and 1.70 ± 0.07 m, respectively. Six normal volunteers were also recruited to this study. They were matched to the chronic renal failure patients notably by age, gender, and size but, most importantly, by daily activity levels, as reported (35). Their mean age, weight, and height were 24 ± 6 yr, 70 ± 6 kg, and 1.71 ± 0.05 m, respectively. All subjects participated in a lengthy, integrated series of experiments that included 1) measurement of maximal leg VO2 during single-leg dynamic knee-extensor exercise; 2) simultaneous proton-MRS measurement of myoglobin desaturation and 31-phosphorus MRS measurements in a similar profile of exercise; and 3) two knee-extensor muscle biopsies, one before and one after a 1-h exercise bout that involved the knee extensors of one leg working at 50% of peak workload. The physiological measurements 1 and 2 above are reported elsewhere (35); the present paper deals with the results of muscle biopsy before and after exercise.

Exercise protocol related to muscle biopsy. Our objective was to obtain muscle samples both before and after a bout of exercise to examine both the structural and functional characteristics of the muscles and their acute response to exercise in terms of angiogenic growth factor gene expression. We chose a 1-h bout of single-leg knee-extensor exercise to mimic the conditions we know from prior studies in the rat (2) and humans (32) to lead to substantial increases in angiogenic growth factor mRNA levels. For the 12 subjects to complete 1 h of exercise, we held the load to 50% of peak power output. This level of exercise used kicking at 60 rpm in both groups, with similar friction belt pan weights of 367 ± 81 g (renal failure patients) and 371 ± 59 g (controls). Muscle biopsy was carried out within 1 h of completing this exercise bout. The preexercise biopsy was obtained on a different day, and in all subjects was taken after absence of exercise had been documented for at least 24 h. Previous work in the rat showed that the VEGF mRNA response to exercise is immediate and is no longer evident 8 h postexercise (2).

Biopsy was performed using local anesthesia for the skin and by sterile technique, accessing the rectus femoris 2–3 cm deep from the skin surface. We used the standard Bergstrom needle aspiration method. About 100–200 mg tissue was obtained in this manner from each biopsy. Puncture wounds were dressed and taped, and all subjects recovered uneventfully.

Of the 12 subjects, one renal failure patient could not be studied because he was transplanted before the biopsy could be scheduled. One of the normal subjects, after completing the physiological portions of the project described above, was unavailable for biopsy, leaving five subjects biopsied in each group.

Disposition of biopsy material. The muscle fragments were each divided into three aliquots that were used for three different analyses: 1) Northern blots for the angiogenic growth factors VEGF, bFGF, and TGF-β1 were prepared from one aliquot; 2) muscle fiber area and capillarity, as well as ultrastructural capillary assessment, were quantified from a second aliquot; and 3) mitochondrial oxidative capacity was assessed from a third aliquot.

Northern blots. We prepared Northern blots using human cDNA probes for the three growth factors, VEGF, bFGF, and TGF-β1, using identical standard methodology as previously reported (2). Total cellular RNA was isolated from each sample by the method of Chomczynski and Sacchi (4). RNA preparations were quantitated by absorbance at 260 nm, and integrity was assessed by ethidium bromide staining after separation by electrophoresis in 6.6% formaldehyde-1% agarose gel. Fractionated RNA was transferred by Northern blot to Zeta probe membrane (Bio-Rad, Hercules, CA). RNA was cross-linked to membrane by ultraviolet irradiation for 1 min and stored at 4°C. The blots were then probed with oligolabeled (α-32P)deoxycytidine triphosphate cDNA probes, which had a specific activity of ≥1×109 disintegrations·min⁻¹·μg DNA⁻¹ (5). The human VEGF probe is a 0.93-kb cDNA fragment isolated from the EcoR I site of pUC-derived plasmid (17). The bFGF probe is a 1-kb Xho I fragment of human bFGF cDNA (16). Prehybridization and hybridizations were performed in 50% formamide, 5× SSC (20× SSC is 0.3 M sodium chloride, 0.3 M sodium citrate), 10× Denhardt’s solution (100× Denhardt’s solution is 2% Ficoll, 2% polyvinyl pyrrolidone), 50 mM sodium phosphate (pH 6.5), 1% SDS, and 250 μg/ml salmon sperm DNA at 37 or 42°C. Blots were washed with 2× SSC and 0.1% SDS at room temperature and 0.1× SSC and 0.1× SDS at 50°C for the VEGF mRNA and at 1× SSC and 0.1% SDS at 60°C for TGF-β1 and bFGF mRNAs. Blots were exposed to XAR-5 X-ray film (Eastman Kodak, New Haven, CT) by use of a Cronex Lightning Plus screen at −70°C. Autoradiographs were quantitated by densitometry within the linear range of signals and normalized to ribosomal 18S RNA levels.

Muscle fiber area and capillarity. Samples were frozen in liquid nitrogen at −130°C and were mounted on a cork, keeping the fibers perpendicular to the cork surface. Serial sections (8 μm thick) were cut in a cryostat maintained at −25°C. Different sections were stained by two different methods. First, a stain for ATPase at pH 9.4 was used to identify the relative amount of type I (oxidative, light stained) and type IIa, IIb, and IIc fibers (nonoxidative, dark stained). Four photomicrographs (×400) were scanned to measure the surface occupied by each type of fiber (NIH Image) (9). A minimum of 300 fibers for each patient was examined. Morphometric measurements of cross-sectional area, perimeter, density, and percentage of each type of fiber were made. Second, a stain for NADH tetrazolium oxidoreductase and simultaneous Ullex Europeus was carried out. NADH allows a clear distinction between type I and type II fibers, whereas Ullex Europeus selectively stains endothelial cells, facilitating the identification of muscle capillaries. Using these techniques, we scanned photomicrographs to calculate two indexes of muscle capillarity, according to muscle fiber type (type I and type II). One was capillary density (number of capillaries/mm² of cross-sectional fiber area).
However, because capillary density depends on fiber size as well as number of capillaries, we also measured the number of capillaries surrounding each fiber.

**Ultrastuctural capillary assessment.** Additional skeletal muscle fragments were fixed in 2.5% glutaraldehyde in phosphate buffer and postfixed in 1% osmium tetroxide. Dehydration in ascending levels of ethyl-alcohol was performed, and tissues were embedded in araldite epoxiresine. Fifty-nanometer ultrathin sections were then cut with introduction of uranyl acetate and lead citrate and examined with a transmission electron microscope (Jeol JEM-1200EX11) (27). Areas showing a transverse section of the capillaries were identified for morphometric analysis. We selected the first 10 capillaries of each slide that had a circular cross-sectional shape and a uniform endothelial cell layer (×5,000 to ×7,500). Thickness of the capillary basal membrane (in nm) expressed as the mean value of six measurements done at 0, 60, 120, 180, 240, and 300 degrees and the percentage of the whole capillary surface area occupied by the capillary basal membrane were assessed (18).

**Mitochondrial oxidative capacity.** Suspensions of pure mitochondria from skeletal muscle were obtained for biochemical assays following standard procedures (3, 26, 34). Therefore, state 3 respiration in the presence of glutamate-malate (complex I substrate) and succinate (complex II substrate) was measured polarographically with a Clark oxygen electrode in a micro-water-jacketed cell (0.25 ml) at 37°C (Hansatech Instruments Limited, Norfolk, UK).

**Statistics and calculations.** Northern Blots were quantified by densitometric methods as previously described (2), and the values were divided by corresponding data for 18S ribosomal RNA to control for lane-loading variation. A two-way repeated-measures ANOVA was used for each of the three growth factors to examine the effects of exercise and renal disease on gene expression. Structural data (fiber cross-sectional area capillary-to-fiber ratio and ultrastructural measurements) were compared between renal failure and normal subjects by unpaired t-test, as were mitochondrial oxidative enzyme activities. Significance was set at \( P < 0.05 \) in each case.

**RESULTS**

**Number of successful studies.** Although 12 subjects (6 with renal failure, 6 control subjects) completed the aforementioned physiological studies, both biopsies (pre- and postexercise) were obtained in just five of each group, as mentioned above. For the molecular studies of growth factor mRNA, only four subjects in each group provided enough material for successful Northern blots in both the resting and postexercise samples. Small samples and RNA degradation prevented complete, paired data from being obtained in the other two. For the metabolic enzyme activity measurements, all six subjects of each group provided material, because this study required only one preexercise tissue sample. For the structural studies of fiber and capillary morphology, all six normal subjects but only five of the renal failure patients provided enough tissue for analysis. The small number of subjects suggests that generalization of the present results be made with caution.

**Northern blots for angiogenic growth factors.** Figure 1 shows the pre- and postexercise Northern blots for VEGF, bFGF, and TGF-\( \beta_1 \) mRNA. We used the 18S ribosomal RNA band to control for lane loading variation in computing the mRNA abundance by densitometry but do not show these bands (for simplicity) in Fig. 1. For VEGF, there is a remarkable increase in mRNA after exercise in both subject groups of about one order of magnitude (\( P < 0.01 \) by ANOVA). Although the numerical values were higher in the renal patients than in the control subjects, ANOVA failed to show significant difference between groups in the exercise response. The densitometry-based mRNA levels are shown in Fig. 2, also for all three angiogenic growth factors. As indicated in Fig. 2, neither exercise nor...
RENAL DISEASE ALTERED THE EXPRESSION OF TGF-β1 OR bFGF mRNA.

Muscle morphometry. Table 1 shows variables relating to fiber type and cross-sectional area and to numbers of capillaries. Fiber type composition was not different between the renal failure patients and normal subjects. Capillary density (i.e., the number of capillaries/mm² of fiber cross-sectional area) was also not different (but, if anything, higher in renal disease associated with a corresponding trend toward smaller fibers). Although the number of capillaries per fiber was not statistically different between the two groups, there were 12% fewer capillaries per fiber in renal failure patients than in healthy subjects. Capillary basement membrane thickness and percentage of capillary area occupied by basal membrane did not differ between the two groups of subjects.

It is worthy of note that from the physiological measurements in these same subjects, muscle O₂ conductance (between red blood cells and mitochondria) was 24% lower (P = 0.03) in the renal failure patients (15). Figure 3 shows how the muscle O₂ conductance relates directionally to the capillary-to-fiber ratio but not at all to capillary density.

Mitochondrial oxidative activities. Figure 4 shows that there were no differences between renal failure patients and normal subjects. These results rule out not only enzyme deficiencies of the complexes constituting the mitochondrial respiratory chain, but also abnormalities in other mitochondrial processes that must be preserved to maintain an effective oxidative phosphorylation (i.e., membrane integrity, ADP-ATP translocase, and coupling of mitochondrial respiratory chain to ATP synthesis).

DISCUSSION

The major findings are that, despite a reduction in muscle capillarity reported in renal failure (and seen in the present study as a trend that correlates with O₂ transport conductance), the increase in VEGF gene expression in response to exercise is not attenuated.

Table 1. Results of muscle morphometric studies

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n = 6)</th>
<th>Chronic Renal Failure Patients (n = 5)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber type composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I, %</td>
<td>33 ± 3</td>
<td>31 ± 3</td>
<td>0.60</td>
</tr>
<tr>
<td>Type II, %</td>
<td>67 ± 3</td>
<td>69 ± 3</td>
<td>0.60</td>
</tr>
<tr>
<td>Perimeter, type I, μm</td>
<td>274 ± 22</td>
<td>266 ± 14</td>
<td>0.79</td>
</tr>
<tr>
<td>Perimeter, type II, μm</td>
<td>310 ± 31</td>
<td>286 ± 17</td>
<td>0.54</td>
</tr>
<tr>
<td>Area, type I, μm²</td>
<td>4,345 ± 635</td>
<td>4,145 ± 412</td>
<td>0.81</td>
</tr>
<tr>
<td>Area, type II, μm²</td>
<td>5,708 ± 961</td>
<td>4,702 ± 404</td>
<td>0.37</td>
</tr>
<tr>
<td>Capillarity indexes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillary density, capillaries mm⁻²</td>
<td>260 ± 8</td>
<td>274 ± 18</td>
<td>0.48</td>
</tr>
<tr>
<td>Capillaries per type I fiber</td>
<td>4.11 ± 0.34</td>
<td>3.70 ± 0.21</td>
<td>0.18</td>
</tr>
<tr>
<td>Capillaries per type II fiber</td>
<td>3.67 ± 0.40</td>
<td>3.16 ± 0.24</td>
<td>0.17</td>
</tr>
<tr>
<td>Overall capillaries per fiber</td>
<td>3.88 ± 0.37</td>
<td>3.44 ± 0.21</td>
<td>0.18</td>
</tr>
<tr>
<td>Capillary basement membrane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness, nm</td>
<td>330 ± 23</td>
<td>375 ± 43</td>
<td>0.36</td>
</tr>
<tr>
<td>% of capillary surface</td>
<td>27.5 ± 21.1</td>
<td>25.1 ± 1.5</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Values are means ± SE.
Additional findings are that neither bFGF nor TGF-β mRNA levels respond to exercise in health or renal failure (whereas in the rat they do (2)). Finally, mitochondrial oxidative activity is not reduced in these renal failure patients.

These data should be considered in light of the physiological findings in the very same subjects studied at a similar time (35). In summary, those findings are that exercise capacity of the quadriceps is reduced compared with normal subjects but that this normalizes when muscle O2 delivery is normalized (achieved by breathing 100% O2). Moreover, over the inspired O2 range from 13% O2 to 100% O2, maximal quadriceps VO2 and power output are linearly proportional to muscle O2 delivery, indicating that maximal VO2 is limited by O2 supply in these patients. In addition, proton MRS (used to measure intracellular Po2) shows that O2 conductance from red blood cells to muscle cells is significantly reduced (by some 24%). All of these physiological findings are reported separately (35).

**Angiogenic growth factor responses in renal failure.** The normal VEGF mRNA response to exercise was the opposite of what we expected to find given published reports of decreased muscle capillarity in renal failure patients (14, 28, 29). To our surprise, the increase in VEGF mRNA represents the highest response in any studies, human, canine, or rat, we have observed to date (2, 7, 33). Although not significantly different from responses in the patients, the normal subjects increased VEGF mRNA less, by sixfold (Fig. 2). As Fig. 1 shows, the responses were extremely consistent and argue that the results are representative despite the being data from only four subjects in each group.

Fig. 3. Structure-function relationships in skeletal muscle. Muscle capillary indexes and O2 transport conductance are shown. Conductance appears related to number of capillaries per fiber (reflecting capillary surface area per fiber) and not to capillary density (reflecting diffusion distance). PTS, patients.

Fig. 4. Selected Krebs cycle oxidative enzyme activities showing no difference between normal subjects and patients with renal failure.
possible explanation for the higher mean increase in renal failure patients is their lower intracellular PO2 as measured by MRS (35). If, as suggested (36), VEGF gene expression is primarily stimulated by intracellular hypoxia, then the lower PO2 found in renal failure patients could account for this, as shown in Fig. 5. Figure 5 suggests that above an intracellular PO2 of ~6 Torr, there would be no exercise-induced VEGF mRNA response (i.e., the exercise-to-rest abundance ratio would be 1.0). This hypothesis is stated with caution, however, because it is based on the assumption that VEGF mRNA abundance is linearly related to intracellular PO2. This cannot be verified from Fig. 5, because there are just two data points.

The failure of either bFGF or TGF-β1 mRNA levels to increase with exercise (in either group) was also surprising given the well-known role for both in angiogenic processes (8, 30, 38). In prior work, we have seen responses of these two growth factors in the normal rat (2), although they are never of the same magnitude as for VEGF. Possibly, in humans, VEGF is the critical angiogenic growth factor in the response of muscle to exercise and bFGF and TGF-β1 are not so involved.

Because multiple, timed biopsies were not justified in this initial study, another possible interpretation is that bFGF and TGF-β1 mRNA levels may not have responded by 30–40 min after exercise, the single time at which biopsies were taken. This is considered unlikely, because VEGF certainly did respond (Figs. 1 and 2) at this time and prior work (2) showed that all three growth factors respond within 1 h of completing exercise of this type, at least in the rat.

Returning to the large increase in VEGF mRNA in the face of probably reduced muscle capillarity, there are several potential interpretations that might be considered. First, despite the trend to lower capillary/fiber counts in the present study, the 12% decrease was not found to be significant, and thus one could argue that the normal gene response and the structural findings are concordant. This would be compatible with the concept that functional blood flow/V\textsubscript{O2} mismatching in skeletal muscle can contribute to the limited capillary O\textsubscript{2} transfer observed in renal patients. Alternatively, on the basis of prior findings of reduced capillarity (14, 28), one might speculate that VEGF is not involved in angiogenesis. This too is considered unlikely given the key angiogenic role for this gene in many other tissues and settings. Thus VEGF gene knockout is embryonically lethal, even in the heterozygous state (6), proving its absolute necessity for normal growth and vascular development. Second, VEGF inhibition by soluble receptor binding or by antibody (1) produces remarkable antiangiogenic effects in tumors and in the eye. Moreover, the absence of any effect of exercise on bFGF and TGF-β1, two well-described growth factors associated with angiogenesis, and the very large mRNA change we saw for VEGF (Figs. 1 and 2) all argue for the probable importance of this growth factor in the exercise response.

Thus we would speculate that our findings may point to some posttranscriptional effects of renal failure. Although transcription, as reflected by increased VEGF mRNA levels (in response to exercise) may be normal, translation to VEGF protein may not be. Of course there are many other steps in the VEGF pathway to angiogenesis; possibly, VEGF receptor number could be reduced in renal failure. From the small tissue samples we were able to obtain and probes available at the time, we were not able to explore these possibilities at present, leaving many questions unanswered.

Structure-function correlations in muscle in renal failure. We and others have argued (10, 13, 37) that O\textsubscript{2} transport between the muscle microvascular red blood cells and the mitochondria does not depend closely on diffusion distance as Krogh (15) originally postulated. Rather, it depends much more on the amount of capillary surface per muscle fiber. This is hypothesized from theoretical (10) and experimental (13, 31) evidence for a large PO2 gradient from red blood cell to the muscle fiber cell surface and essentially no gradient within the generally longer distances from the cell membrane to the mitochondria. This hypothesis would predict that muscle O\textsubscript{2} conductance depends on capillary number (per fiber) much more than on capillary density. The former reflects capillary surface area, the latter more closely reflects diffusion distance for O\textsubscript{2}, because it is directly related to muscle fiber cross-sectional area. Figure 3 provides additional evidence in support of this hypothesis, because physiologically measured O\textsubscript{2} conductance (35) relates well to capillary number per fiber but not to capillary density in these patients and subjects.

Furthermore, the data of Fig. 3 taken together with previous reports of reduced capillarity in renal failure (14, 28) suggest that the lack of statistical significance in the capillary/fiber data, despite the 12% lower values in renal failure, is likely a type II error. Unfortunately, the resources required to add subjects to this multidisciplinary project no longer exist.
It is possible that one contributing factor to reduced O$_2$ conductance other than capillarity per se is extent and thickness of the capillary basement membrane. However, Table 1 shows no differences in parameters of the basement membrane between groups. Thus we suggest that basement membrane structure does not explain the reduced muscle O$_2$ conductance in the present group of renal failure patients.

**Mitochondrial oxidative activity.** The present data (Fig. 4) show normal mitochondrial oxidative activity. This is consistent with the physiological data from the same subjects showing normal peak VO$_2$ of the quadriceps when muscle O$_2$ transport is normalized by breathing 100% O$_2$ (35). It is further consistent with $^{31}$P-MRS data on phosphocreatine recovery rates after exercise (35), again from the same subjects, which show no differences from normal. It should be noted that our control subjects were matched not only for age, size, and gender but also for a sedentary lifestyle. Consequently, multidisciplinary data reflecting physiological, magnetic resonance-based, and biochemical measurements made at different times but in the same subjects all point to normal biochemical capacity for oxidative metabolism during exercise. The present renal failure patients were all young (mean age 25 yr, range 19–34), free of other systemic disease, and intensively cared for by three times weekly hemodialysis while awaiting renal transplantation. Furthermore, all had increased hematocrits (compared with untreated patients with renal failure) as a consequence of erythropoietin therapy. Thus our subjects may well exemplify optimal clinical conditions, and older patients with, perhaps, atherosclerosis, peripheral vascular disease, and long-standing hypertension, may be very different.

In conclusion, we found no difference from normal in gene expression of angiogenic growth factors (VEGF, bFGF, and TGF-β$_1$) after exercise in patients with renal failure. This is despite some reduction in muscle capillarity and muscle O$_2$ transport conductance, and we postulate that there may be a posttranscriptional abnormality in the VEGF-mediated pathway to angiogenesis to explain the discrepancy between normal gene expression and the morphological and functional differences. On the other hand, multidisciplinary measures of mitochondrial oxidative activity are internally consistent and not different from normal and support the hypothesis that O$_2$ transport defects, rather than metabolic abnormalities in muscle, contribute to exercise limitation in well-managed, young patients with chronic renal failure.

**Perspectives**

Interest in the systemic effects of chronic diseases such as emphysema, chronic heart failure, and chronic renal failure is currently high, and the extent to which skeletal muscle in particular may be affected is important as a determinant of quality of life. If mechanisms of muscle abnormalities can be discovered, this could eventually lead to specific therapy, perhaps even at the level of the gene. The relatively easy access to muscle tissue by biopsy coupled with modern molecular approaches offers the promise of uncovering such pathways. Prior work has shown that muscle O$_2$ transport from red blood cell to mitochondria is affected more by muscle capillarity than by other potential impediments, providing the stimulus to the present work. On the presumption that VEGF is a key growth factor without which capillarity cannot be sustained (as suggested by anti-VEGF studies arresting cancer growth), the present paper has begun a search for the basis of reduced capillarity in renal failure. Although we suggest herein that transcriptional regulation of VEGF in response to exercise is not impaired, there are many points downstream of VEGF gene expression that remain to be explored as potential explanations. These include VEGF translation, receptor abundance, and signaling, among others. It should also be remembered that VEGF is not the only molecule important to angiogenesis. Although in this study we saw little apparent involvement of either bFGF or TGF-β$_1$ at the level of gene expression, these and other substances with angiogenic activity may also need to be evaluated, possibly by gene array technology.

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