Abstract. Although angiogenic therapy using recombinant growth factors holds much hope for the treatment of ischemic diseases, there are still unanswered questions including the method, doses or duration of therapeutic approach. We evaluated the angiogenic effects of vascular endothelial growth factor (VEGF) on rat heart and gastrocnemius muscles when this was administered intramuscularly and compared them to those obtained from rats, which exercised daily. Conclusion: Both daily swimming exercise and intramuscular administration of VEGF increased angiogenesis in rat heart, even though exercise alone was the only one that increased angiogenesis quite significantly. The combined protocol (administration of growth factor and exercise) led to an increase of angiogenesis in cardiac muscles. In contrast, there was no effect on the lateral gastrocnemius muscle either by VEGF or exercise, whereas these together induced angiogenesis locally at the site of injection.

Angiogenesis is the formation of new blood vessels from pre-existing vessels (1). In adults, angiogenesis is essential for the female reproductive cycle and for repair remodeling and regeneration of tissues, as in wound healing. Furthermore, muscles under exercise undergo changes leading to their growth, a process associated with angiogenesis (2). Neovascularization also plays a major role in pathological processes such as tumor growth, metastasis and rheumatoid arthritis (3). Over the years, the mechanisms underlying the angiogenic process have attracted attention and accumulated evidence suggests that the angiogenic process is principally regulated by a variety of hormonal and growth factors. The use of recombinant growth factors like vascular endothelial growth factor (VEGF) for therapy in clinical conditions associated with ischemia represents a hopeful strategy for blood flow restoration. The VEGF family includes several members: VEGF, placenta growth factor (PlGF), VEGF-B, VEGF-C, VEGF-D (4, 5). VEGF-mRNA is induced by hypoxia in cultured cells and up-regulated in tumor cells adjacent to necrotic areas, as revealed by in situ hybridization. As a secreted endothelial cell-specific mitogen, VEGF is also a candidate for ischemia-induced angiogenesis in pathophysiological conditions in tissues (6).

Unlike the fibroblast growth factor (FGF) family, VEGF is a more specific mitogen for endothelial cells and has the advantage of being secreted by intact cells. Therefore, VEGF may be superior to FGF as a therapeutic agent. Gene transfer is also being widely investigated to determine its applications to angiogenesis. Although angiogenic therapy using recombinant growth factors holds much hope for the treatment of ischemic diseases, many questions still remain unanswered including the method of administration, the appropriate dose of these factors or the duration of the therapeutic approach.

This present study aimed at evaluating the local and systemic angiogenic effects of VEGF on intramuscularly administered heart and skeletal muscle and exercise.

Materials and Methods

Thirty-five Wistar male rats of 330-380 g body weight were used for this study. The animals lived under stable conditions of temperature, reverse light cycle program, and were allowed to eat ad libitum. They were divided into four groups:

Group A consisted of 14 rats, which were used as controls. This was subdivided into group A1 consisting of 7 rats, who received no treatment, while 7 rats in group A2 were administered intramuscularly, under ether anesthesia, 0.1ml saline to the right gastrocnemius every three days, for a total period of 15 days.
**Table I. Mean number of heart vessels per optical field and mean values of body weight, heart weight and weight of the right and left gastrocnemius muscles in the studied groups.**

<table>
<thead>
<tr>
<th>Mean number of vessels (p.o.f.)</th>
<th>Body weight (g)</th>
<th>Heart weight (g)</th>
<th>Weight of right gastrocnemius (g)</th>
<th>Weight of left gastrocnemius (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A1 35.40 ± 10.20</td>
<td>324.46 ± 19.65</td>
<td>1.47 ± 0.17</td>
<td>2.13 ± 0.15</td>
<td>2.14 ± 0.26</td>
</tr>
<tr>
<td>Group A2 36.00 ± 10.80</td>
<td>325.50 ± 18.11</td>
<td>1.49 ± 0.12</td>
<td>2.18 ± 0.14</td>
<td>2.11 ± 0.12</td>
</tr>
<tr>
<td>Group B 53.80 ± 11.50 a</td>
<td>359.89 ± 32.02</td>
<td>1.40 ± 0.14</td>
<td>2.67 ± 0.23 b</td>
<td>2.61 ± 0.26 b</td>
</tr>
<tr>
<td>Group C 41.20 ± 6.61</td>
<td>378.25 ± 23.70</td>
<td>1.39 ± 0.18</td>
<td>2.62 ± 0.12 a</td>
<td>2.52 ± 0.24 a</td>
</tr>
<tr>
<td>Group D 49.60 ± 6.61 a</td>
<td>382.74 ± 24.10 a</td>
<td>1.21 ± 0.18</td>
<td>2.60 ± 0.17 a</td>
<td>2.55 ± 0.16 a</td>
</tr>
</tbody>
</table>

*p<0.05 in comparison to the controls

Results

The angiogetic effects of exercise and VEGF on cardiac muscle. No difference in angiogenesis in heart was found between groups A1 (35.4±10.2) and A2 (36±10.8) (Table I), (Figure 1A). In group B, the mean number of vessels was 53.8±11.5 p.o.f. (Table I). Comparing the number of vessels of groups A1 and B, it is clear that exercise significantly increased the heart vasculature (p<0.05).

In group C, where the VEGF factor was administered intramuscularly, the mean number of vessels in heart was 41.20±6.61 (Table I), which is not significantly different from control group A2.

On the contrary, in group D (administration of growth factors and daily exercise), the mean number of heart vessels was significantly greater than in group A1 or group A2 (Table I), (Figure 1B). None of the other comparisons showed any significant differences (p>0.05) (Table I).

Finally, both daily exercise and intramuscular administration of VEGF were shown to increase angiogenesis in rat heart, even though exercise alone was the only one that increased angiogenesis. The combined protocol (administration of growth factor and exercise) led to an important increase in angiogenesis in heart, in comparison with controls, but there was no significant difference compared to rats undergoing exercise alone.

**The effects of exercise and VEGF on cardiac muscle growth.**

The cardiac muscle weight was determined at the end of the experiment as an index of muscle growth. Table I illustrates that there were no statistically significant differences in mean heart weight±SD among the various groups.
The effects of exercise and VEGF on angiogenesis in gastrocnemius muscles. No difference in angiogenesis in gastrocnemius muscles was observed between controls i.e. untreated and saline-treated controls (Table II), (Figure 1C).

In group B (rats under exercise), the mean number of vessels in the right and left gastrocnemius muscles was 12.82±2.8 and 12.7±3.0, respectively. Comparison between groups A1 and B revealed a significant reduction in the number of vessels in both right (p<0.05) and left gastrocnemius muscles (p<0.05). On the other hand, the mean muscle weights of group A and group B (Table I) demonstrated a statistically significant increase in the left (p<0.01) and right (p<0.01) exercising muscle, respectively.

With regard to the effects of VEGF on gastrocnemius muscles (group C), the mean number of vessels differed between the right gastrocnemius, where the factor was injected (intramuscularly), and the left gastrocnemius muscle (Table II). Thus, in the right muscle the mean number of vessels was 29.20±2.68, while in the left muscle it was 19.40±5.02. Comparison of group C with controls (group A2) with regard to the number of vessels in the right and the left gastrocnemius revealed that intramuscular administration of VEGF increased vascularisation significantly (p<0.05) and selectively in the right gastrocnemius muscle (Table II), (Figure 1D). There was a slight increase in angiogenesis in the left gastrocnemius, but this was not statistically significant. Furthermore, the mean number of vessels of group C in the right as well as in the left muscle was significantly higher, than in group B (p<0.005 and p<0.05, respectively).

The combination of administration of VEGF and exercise (group D) had different effects on the right (22.20±2.48) and the left (17.63±1.58) gastrocnemius muscles (Table II). Comparison of the number of vessels in the right and the
left gastrocnemius with controls (group A2) showed that combined administration of VEGF along with daily exercise significantly increased vascularization in the right gastrocnemius muscle \( (p<0.05) \), but not in the left muscle. On the other hand, the number of vessels in the right and the left muscle of group D was significantly higher than those of group B consisting of swimming rats \( (p<0.005 \text{ and } p<0.05, \text{ respectively}) \). However, comparison of the combined protocol (group D) with the administration of VEGF alone (group C) did not show any significant differences regarding either the left or the right gastrocnemius muscle.

The effects of exercise and VEGF on the growth of the gastrocnemius muscles in rat. The weights of the right and the left gastrocnemius muscles were also determined as an index of muscle growth (Table I). Exercise (group B) significantly increased the weight of the right and left \( (p<0.01) \) gastrocnemius muscles compared to controls. A statistically significant increase in the weight of both the right \( (p<0.05) \) and the left gastrocnemius muscles \( (p<0.05) \) was also observed in group C, when compared to controls. Similarly, in group D the increase in the mean weight of the right \( (p<0.05) \) as well as the left gastrocnemius muscle \( (p<0.05) \) was statistically significant compared to controls.

### Discussion

Despite the fact that a large number of cytokines have been recognized to cause neovascularization, VEGF and FGFs have presented the best results in experimental models and clinical trials (10). However, there are still many unanswered questions. Symes (11) in a review article concluded that angiogenic therapy by VEGF in 165 patients combined with coronary bypass improved angina, treadmill exercise tolerance and myocardial perfusion. The "Traffic" study (12) also illustrated an improved exercise capacity in patients with intermittent claudication, without any statistical difference regarding the way the growth factor was administered (one or two doses). Clinical studies in patients with coronary insufficiency exhibited an increase in exercise training and provided objective evidence of improved perfusion and left ventricular function (13, 14). Studies have also reported increased angiogenesis in heart induced by conditions such as exercise training, dipyridamole or thyroid hormone therapy (15, 16).

When we administered VEGF intramuscularly to rats to the right gastrocnemius muscle, neovascularization in the myocardium was observed. This effect was important, since the angiogenic factor was administered intramuscularly to the right gastrocnemius muscle, while until now all the relative reports mention that the angiogenic factor was effective when administered either intravenously or locally to the myocardium. Moreover, exercise significantly induced neovascularization in the myocardium of rats. The best results regarding neovascularization in rat heart were observed in the group combining administration of VEGF and exercise.

Current evidence demonstrates that VEGF protein levels elevate at capillary sites in skeletal muscles during exercise and, even though the type of capillary growth remains to be determined, the most likely stimuli for angiogenesis are increased blood flow and shear forces to vessels supplying the active fibres, probably linked with metabolic factors (17). Other investigators also suggest that angiogenesis is regulated through a balancing mechanism between stimulatory and inhibitory factors (18), while exercise can up-regulate angiogenic factors, one of which may be VEGF. A major stimulus for augmented secretion of angiogenic factors is hypoxia (19). Similar conclusions have also been reported by others, detecting increased levels of VEGF-mRNA in skeletal muscles after exercise in rats (20, 21) and in humans (22, 23). Amaral et al. (24, 25) suggested that VEGF and angiotensin-2 play an important role in exercise-induced angiogenesis, as demonstrated by experiments involving electrical stimulation or short-term exercise of skeletal muscles.

Intramuscular administration of the right gastrocnemius muscle exhibited a local angiogenic effect. This means that VEGF was as effective on skeletal muscle as on the heart. However, its effect on skeletal muscle was local, i.e. it was not obvious anywhere else apart from the injected site. This suggests that the angiogenic threshold between skeletal and heart muscle is different, explaining why the circulating levels of VEGF were capable of inducing angiogenesis in heart but not in skeletal muscle. Although angiogenesis is an adaptive response to exercise training in skeletal muscle, in our study it seems that exercise had a negative effect. Future studies involving comparisons of the effects of various degrees and duration of exercise with those induced by different doses of VEGF might help in understanding this issue better.
In conclusion, this study has shown that intramuscular administration of VEGF to rat in vivo leads to neovascularization of the heart compared to exercise. Furthermore, it is also effective in high concentrations on skeletal muscle but only locally. These findings are interesting regarding the potential administration of VEGF to alleviate conditions characterized by insufficient blood supply, such as limp and ischemia. To date, the existing experience is limited to the intracardiac or intravenous administration of angiogenic factors. This study shows, for the first time, the local and systemic beneficial effects of VEGF when administered intramuscularly to rat, providing a basis for further application of this factor to humans, so as to treat ischemic vascular lesions.

References