Physical Conditioning Decreases Norepinephrine-Induced Vasoconstriction in Rabbits
Possible Roles of Norepinephrine-Evoked Endothelium-Derived Relaxing Factor

Hsiun-ing Chen, PhD; Hsing-Tan Li, MS; Chien-Chih Chen, BS

Background  Physical activity can reduce sympathetic tone and may be beneficial to human health. Whether the vascular responses to norepinephrine (NE), an adrenergic vasoconstrictor, could be altered by chronic exercise was unclear. We therefore conducted this study to investigate the effects of endurance exercise training on NE-induced vasoconstrictive responses in healthy rabbits. Possible mechanisms were also studied.

Methods and Results  Twenty-four male New Zealand White rabbits were used for this study. They were divided into two groups: control and training. The training group was trained on a treadmill with running speed of 0.88 km/h at a 0° grade for 10 to 60 minutes per day, for 5 days a week for a total of 8 weeks. At the end of the experiments, thoracic aortae (3 mm long) were isolated. The vascular tension was measured with a force transducer. The dose-response relation of NE-induced vasoconstriction was determined and compared for control (n=5) and trained (n=6) groups. To verify the possible involvement of endothelium-derived relaxing factor (EDRF) in the alteration of NE-induced vasoconstriction after exercise training, we compared the vascular responses to NE in endothelium-intact, N\(^{-}\)nitro-L-arginine (L-NNa, 10\(^{-4}\) mol/L)—pretreated, or denuded vessel segments (n=4 for each experiment of each group). EDRF release in the presence or absence of NE was also evaluated by the increased tension induced by hemoglobin (10\(^{-2}\) mol/L), an EDRF scavenger (n=6 for the control group and n=8 for the trained group). In addition, vascular responses to some specific adrenergic agonists (ie, phenylephrine, an \(\alpha\)-agonist, and clonidine, an \(\alpha\)-agonist) were also studied to see if a specific adrenergic receptor was involved (n=4 for each experiment of each group). Our results indicated that (1) [NE\(_{EDR}\)] of the thoracic aorta was elevated by exercise training; (2) in the presence of NE, EDRF release from the thoracic aorta, assessed by addition of hemoglobin or L-NNa, was higher in the trained group than in the control group; (3) both phenylephrine (10\(^{-4}\) mol/L) and clonidine (10\(^{-5}\) mol/L) could evoke vasorelaxation that would be inhibited by L-NNa; and (4) in addition to causing vasoconstriction, NE could stimulate EDRF release, possibly via \(\alpha\)- and \(\alpha\)-receptors of endothelial cells.

Conclusions  Our data suggest that exercise training may decrease NE-induced vasoconstrictive response and may increase NE-stimulated EDRF release. (Circulation. 1994;90:970-975.)

Key Words  exercise • adrenergic agents • relaxing factors • norepinephrine

The vascular endothelium not only serves as a diffusion barrier but also plays an important modulatory role in the response of vascular smooth muscle to a variety of stimuli. In 1980, Furchgott and Zawadzki\(^1\) discovered that the vascular response to acetylcholine was strongly dependent on the presence of the endothelial cell layer. Their findings suggest the existence of a mediator passing from endothelial cells to vascular smooth muscle. The mediator, which causes vasorelaxation and is not blocked by cyclooxygenase inhibitors, has been called endothelium-derived relaxing factor (EDRF), and its characteristics are similar to those of nitric oxide (NO).\(^2\) Since then, it was noticed that endothelial cells exert an important role in the modulation of local vascular tone by the release of endothelium-dependent relaxing and constricting factors.\(^3,4\) However, the net balance between dilatory and constricting signals is disturbed under pathophysiological conditions. Previous studies have indicated that vasoconstrictive response is dramatically enhanced and endothelium-dependent relaxation is significantly impaired in hypertension and atherosclerosis.\(^5-9\) The increased vasoconstrictive response and the decreased vasorelaxation may be the principal underlying mechanisms responsible for vasospasm and high vascular resistance in pathological conditions.\(^10,11\)

According to clinical, epidemiological, and pathological studies, physical activity appears to play an important role in the treatment and prevention of several cardiovascular diseases.\(^12-19\) However, its underlying mechanisms are unclear. Previous human studies demonstrated that regular exercise could reduce sympathetic tone\(^20\) and that prolonged infusion of norepinephrine (NE) could depress the pressor response to NE.\(^21\) Some in vivo animal studies also showed that chronic exercise could attenuate baroreflex and pressor responses to adrenergic agents.\(^23,24\) Based on the above studies, we hypothesized that chronic exercise may reduce vasoconstrictive response and/or enhance endo-

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thelium-dependent vasorelaxation. In a previous study, we have shown that acetylcholine-induced vasorelaxation due to EDRF release was increased by endurance exercise training in healthy male New Zealand White rabbits.\(^{25}\) In 1981, Wiegman et al\(^{26}\) reported that exercise training decreased the cremaster arteriole sensitivity to NE-induced vasoconstriction in rats. In contrast, an in vivo study of DiCarlo et al\(^{27}\) showed that 4 weeks of daily walking exercise in dogs significantly enhanced the coronary vascular sensitivity to NE. Although Edwards et al\(^{28}\) found that the trained hypertensive rats exhibited a trend for lower maximal contractile response to NE, they did not find any difference in dose response of NE between control and trained animals. Therefore, whether NE-induced vasoconstriction was changed by exercise training was still unclear, and we conducted this study to investigate the effects of endurance exercise training on NE-induced vasoconstrictive response in the isolated thoracic aorta of healthy rabbits. Possible mechanisms were also studied.

**Methods**

**Animals and Surgery**

This study was conducted in conformity with the policies and procedures detailed in the "Guide for the Care and Use of Laboratory Animals." Twenty-four male New Zealand White rabbits were fed a standard diet and were housed in an environmentally controlled room. These animals weighed 0.8 to 1.2 kg at the beginning of the experiments (ie, about 1 month of age), and they grew up to weigh 2.7 to 3.5 kg (about 3 months old) by the end of experiments. The animals were anesthetized with ketamine (30 mg/kg IV) and sodium pentobarbital (20 mg/kg IV) via the marginal ear vein. Under anesthesia, they were implanted with ECG electrodes subcutaneously. After a recovery period of 1 week, they were randomly assigned to either the control or training group. Heart rates of the awake animals were monitored on a polygraph (Gould 2200S recorder) through an ECG/Biostach amplifier (Gould). Because some of the implanted ECG electrodes were loosened or disconnected during the later period of experiments, heart rates were analyzed for those that were successfully monitored at the end of experiments.

**Exercise Training Protocols**

A training protocol similar to that of our previous study\(^{29}\) was used. After 1 week of familiarization, the training animals ran on a leveled treadmill (model Q55, Quinton Instruments Co) at the speed of 0.88 km/h for 10 minutes for the first week. On subsequent training weeks, the running time was extended 5 to 10 minutes each week until they ran for 60 minutes per day. They were trained for 5 days per week for a total of 8 weeks. The training intensity was approximately 70% of their maximal exercise capacity, which was estimated from heart rates. In contrast, the sedentary control rabbits were placed on the treadmill for 10 minutes each day but did not receive any exercise training. During the training period, resting heart rates of the awake animals were monitored weekly after 30 minutes of rest.

**Preparation of Vessel Segments**

The animals were killed while under general anesthesia with ketamine (30 mg/kg IV) and sodium pentobarbital (20 mg/kg IV) at least 48 hours after training to avoid acute effects of exercise. All animals were killed within the same time period to avoid diurnal variation. Rings of thoracic aorta (3 mm long) were carefully excised and submerged in organ chambers containing oxygenated Krebs-Ringer solution gassed with 95% O\(_2\)-5% CO\(_2\) (37°C, pH 7.4). This solution had the following composition (in mmol/L): 118.0 NaCl, 4.8 KCl, 2.5 CaCl\(_2\), 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 24 NaHCO\(_3\), 0.03 Na\(_2\)-EDTA, and 11.0 glucose. In addition, indomethacin (10\(^{-5}\) mol/L) was added into the solution to prevent the formation of endogenous prostaglandins. At the end of each experiment, the surface areas of vessel segments were measured after longitudinal opening for the normalization of vascular tension. There was no significant difference in the surface areas of vessel rings between control and trained groups (0.429±0.031 versus 0.434±0.023 cm\(^2\), respectively).

The rings were suspended in organ chambers by two platinum stirrups. One stirrup was anchored by a tissue holder in the organ chamber, and the other was connected to a force transducer (Grass FT-03, Grass Instruments Co) for the recording of isometric tension on a polygraph (Gould). These vessel rings were then progressively stretched to their optimal passive tension, ie, 9g, at which the contractile response evoked by NE was maximal. In the experiments using endothelium-intact vessel segments, functional integrity of endothelium was confirmed by the fact that acetylcholine could induce at least 80% of vasorelaxation in NE-preconstricted vessel rings. The integrity of vascular endothelium was proved by silver stain at the end of each experiment.\(^{29}\) In the experiments that used denuded vessel segments as the specimens, the endothelium of vessel rings was removed by careful rubbing of the inner surface of vessel segments with a roll of filter paper. In addition, the removal of endothelium was functionally confirmed by the absence of acetylcholine-induced vasorelaxation in NE-preconstricted vessel segments. After optimal passive tension had been obtained, the vessel rings were equilibrated for 120 minutes, and the following experiments were executed.

**Comparison of Dose-Response Relation of NE-Induced Vasoconstriction Between Control and Trained Groups**

After preparing an endothelium-intact vessel segment for each animal as described above, the dose response of NE-induced vasoconstriction was determined by adding NE accumulatively into the chamber solution. The final concentrations of NE in the chamber were from 10\(^{-8}\) to 10\(^{-5}\) or 10\(^{-7}\) mol/L. The vascular sensitivity to NE was evaluated by its median effective dose of NE (D\(_{50}\), NE\(_{50}\)), which was obtained by curve fitting of the semilog dose-response relation for each vessel segment.

**EDRF Release in the Presence or Absence of NE**

To investigate whether NE can induce EDRF release in addition to its vasoconstrictive effect, dose-response relations of NE-induced vasoconstriction were performed under the following three conditions: (1) endothelium-intact but without N\(^{\text{N}}\)-nitro-L-arginine (L-NNA) pretreatment (+E), (2) endothelium-intact with L-NNA (10\(^{-4}\) mol/L) pretreatment for 15 minutes (+L-NNA) on the same vessel rings as in condition 1, and (3) denuded (−E). To compare between control and trained groups the vasoconstrictive responses to NE under these three situations, ie, the contractile force (normalized by surface area) produced by 10\(^{-7}\) mol/L of NE in the above conditions, was also measured.

At 9g of optimal passive tension, the basal release of EDRF in each endothelium-intact vessel ring was determined by the increased tension with addition of hemoglobin (10\(^{-7}\) mol/L), which is a scavenger of EDRF. Similar experiments were also performed on vessel segments preconstricted by NE (10\(^{-7}\) mol/L).

**Vascular Responses to Specific Adrenergic Agonists in NE-Preconstricted Vessel Segments**

To study whether a specific adrenergic receptor was involved in the training effect on vascular responses to NE, we
compared vascular responses to a specific $\alpha_1$-agonist, phenylephrine ($10^{-8}$ mol/L), and to a specific $\alpha_2$-agonist, clonidine ($10^{-8}$ mol/L), in NE ($10^{-7}$ mol/L)-preconstricted endothelium-intact vessel rings, with or without treatment of L-NNA, for control and trained groups.

**Dose-Response Relation of Phenylephrine-Induced Vasoconstriction**

In addition to NE, we would like to know if exercise training could alter the vascular response to other vasoconstrictors. Therefore, in some experiments we also evaluated the dose-response relation of phenylephrine (from $10^{-7}$ to $2 \times 10^{-6}$ mol/L), a vasoconstrictor acting on the $\alpha_1$-receptor of vascular smooth muscle cells.

**Reagents**

All chemicals for the preparation of Krebs-Ringer solution were purchased from Merck. Other reagents were purchased from Sigma Chemical Co.

**Statistical Analysis**

All data were expressed as mean±SEM. Vascular responses to NE in the thoracic aorta under three above-mentioned conditions—(1) endothelium-intact but without L-NNA pre-treatment, (2) endothelium-intact with L-NNA pretreatment, and (3) denuded—were compared by ANOVA. Unpaired Student's $t$ test was used to compare the results of two studied groups (ie, control versus trained) or the results between endothelium-intact and denuded vessel segments. EDRF release and NE-induced vasoconstriction with or without L-NNA pretreatment were analyzed by paired Student's $t$ test because these experiments were performed in the same vessel segments. Differences would be considered significant at $P<.05$. All statistical analyses were executed by running the statistical software package SPSS-PC+.

**Results**

**Resting Heart Rates**

After endurance exercise training, the trained rabbits had significantly lower resting heart rates than the controls (Table 1; $n=4$ and $n=5$, respectively). This finding indicated that our training protocol did have training effects.

**Comparison of Dose-Response Relation of NE-Induced Vasoconstriction Between Control and Trained Groups**

In this part of the experiments, we found that the dose-response curve of NE in the trained group ($n=6$) shifted to the right when compared with the controls ($n=5$) (Fig 1). Although the vascular sensitivity to NE-induced vasoconstriction in the trained group (indicated by its $ED_{50}$) was decreased, the maximal contractile force induced by NE and normalized by the surface area of the vessel rings in this group did not differ from that of the controls (Table 2).

**TABLE 1. Resting Heart Rates Before and After Training**

<table>
<thead>
<tr>
<th>Group (n=5)</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>226±6</td>
<td>212±7</td>
</tr>
<tr>
<td>Trained</td>
<td>229±3</td>
<td>184±5*</td>
</tr>
</tbody>
</table>

bpm indicates beats per minute. *$P<.05$ (before vs after exercise training).

![Fig. 1](image-url)

**TABLE 2. Comparison of Sensitivity, Indicated as $ED_{50}$, and Maximal Active Tension of NE-Induced Vasoconstrictive Response in Thoracic Aorta Between Control and Trained Groups**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=5)</th>
<th>Trained (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$ED_{50}$ ($-\log M$)</td>
<td>7.14±0.06</td>
<td>6.73±0.14*</td>
</tr>
<tr>
<td>Maximal contraction, g/cm²</td>
<td>21.1±1.2</td>
<td>19.0±1.6</td>
</tr>
</tbody>
</table>

Maximal contraction indicates maximal contractile tension per unit surface area; NE, norepinephrine. *$P<.05$ (control vs trained).
endothelium or pretreatment of L-NNA on the vessel segments could enhance the contractile response to NE in the trained group (P<.05), these responses were not significantly different from those in the control group.

The results of EDRF release, as evaluated by the hemoglobin-induced tension increase, in the absence or in the presence of NE are shown in Fig 3 (n=6 and 8 for control and trained groups, respectively). It was noticed that EDRF release in the presence of NE was much greater than its basal release (without NE) in the trained group. EDRF release of the thoracic aorta in the presence of NE was higher in the trained group than that in the controls.

Vascular Responses to Specific Adrenergic Agonists in NE-Preconstricted Vessel Segments

Because our results implied that NE might induce EDRF release and that exercise training could enhance this effect (Figs 2 and 3), the vascular responses to specific adrenergic agonists were further investigated to clarify whether these effects of NE were through a specific adrenergic receptor on endothelial cells. We found that either phenylephrine (10^-4 mol/L), a specific α1-agonist, or clonidine (10^-4 mol/L), a specific α2-agonist, could induce a vascular relaxing effect on NE-preconstricted vessel segments and that these relax-

![Image](https://via.placeholder.com/150)

Fig 3. Bar graph of endothelium-derived relaxing factor (EDRF) release with or without norepinephrine (NE) in thoracic aorta. The abscissa represents the increased tension due to the addition of hemoglobin (10^-5 mol/L), presumably blocking the effect of EDRF on vasorelaxation. **P<.01 (paired t test; trained with NE vs trained without NE); #P<.05 (unpaired t test; control with NE vs trained with NE). The sample sizes (n) were equal to 6 and 8 for control and trained groups, respectively.

![Image](https://via.placeholder.com/150)

Fig 4. Bar graph of comparison of phenylephrine (10^-8 mol/L) evoked vasorelaxing responses in norepinephrine-preconstricted thoracic aortae between control and trained groups. PHE indicates addition of phenylephrine; PHE+L-NNA, phenylephrine-induced response in N^ε-nitro-L-arginine (10^-7 mol/L)-pretreated vessel segments. **P<.05 (control vs trained); *P<.05 (PHE vs PHE+L-NNA in the same group). n=4 for each group.

![Image](https://via.placeholder.com/150)

Fig 5. Bar graph of comparison of clonidine (10^-6 mol/L) evoked vasorelaxing responses in norepinephrine-preconstricted thoracic aortae between control and trained groups. CLO indicates addition of clonidine; CLO+L-NNA, clonidine-induced response in N^ε-nitro-L-arginine (10^-7 mol/L)-pretreated vessel segments. **P<.05 (control vs trained); *P<.05 (CLO vs CLO+L-NNA in the same group). n=4 for each group.
weeks of endurance training; (2) EDRF release in the presence of NE was higher than its basal release in the trained group; (3) in the presence of NE, thoracic aortae of the trained group had greater EDRF release than the control group; (4) the vascular sensitivity to NE-induced contraction was enhanced by either removal of the endothelium or pretreatment of L-NNA on the vessel segments; (5) exercise training could reduce the vasoconstrictive response to $10^{-5}$ mol/L of NE, and this training effect disappeared after denudation of the endothelium or pretreatment of L-NNA on the vessel segments; (6) in NE-constricted vessel segments, phenylephrine ($10^{-4}$ mol/L) and clonidine ($10^{-5}$ mol/L) induced vasorelaxation that could be inhibited by L-NNA treatment; (7) the relaxing effects of phenylephrine and clonidine were greater in the trained group than in the controls; and (8) no significant difference in the phenylephrine-induced vasoconstrictive response was found between control and trained groups.

Several studies have shown that chronic exercise may reduce sympathetic tone and suppress the pressor response to adrenergic agents. In addition to the systemic adrenosympathetic adaptation to chronic exercise, the possibility that alteration of local modulation in vascular tone by chronic exercise should not be ruled out. In fact, our previous study showed that exercise training could enhance acetylcholine-induced vasorelaxation due to EDRF release on one hand. The present study, on the other hand, showed that the sensitivity of NE-induced vasoconstrictive response, indicated as $[\text{NE}]_{ED_{0.5}}$, was reduced after exercise training. Our findings indicate that the thoracic aortae of normal rabbits are not only more sensitive to endothelium-dependent vasodilators but also less responsive to NE, an adrenergic vasoconstrictor, after endurance exercise training. These findings were apparently contradictory to the reports of DiCarlo et al. who demonstrated that exercise training increased coronary vasoconstrictor responses to NE in vivo in dogs and that training did not affect the dose response of NE in rats. This discrepancy might be due to differences in the studied vessel types, the experimental design, or the animal models used.

In this study, we found that NE could induce EDRF release and therefore might reduce its vasoconstrictive effect (Figs 2 and 3). This NE-induced EDRF release was not caused by the greater tension produced by NE, because our preliminary study demonstrated that basal EDRF release was not increased by higher passive tension (data not shown). The observation of NE-induced EDRF release in our study was consistent with some previous reports.

Because adrenergic agents can evoke endothelium-dependent vasorelaxation in addition to the vasoconstrictive effect, exercise training in this study may enhance NE-induced EDRF release that compromises the vasoconstrictive response caused by NE. Indeed, we found that EDRF release in the presence of NE was significantly higher than in the controls after training, although the basal release of EDRF in the trained group was similar to that in the controls (Fig 3). We also observed that the reduced sensitivity of NE due to exercise training was eliminated by denudation of the endothelium or by pretreatment of L-NNA, an NO synthase inhibitor (Fig 2).

Previous studies have suggested that adrenergic agents may stimulate EDRF release via endothelium-dependent $\alpha_1$- or $\alpha_2$-adrenoceptor, which may vary with different vessels of different animals. Therefore, it is possible that exercise training in rabbits may, at least in part, decrease NE-induced vasoconstriction due to enhancing NE-induced EDRF release via upregulating endothelial $\alpha$-adrenoceptors. In the experiments of phenylephrine (a specific $\alpha_1$-agonist) and clonidine (a specific $\alpha_2$-agonist)-induced vasorelaxing responses of the studied vessel rings, we observed that exercise training might enhance $\alpha_1$- or $\alpha_2$-receptor-mediated EDRF (or NO) release from endothelial cells (Figs 4 and 5).

Another possibility for the reduced responsiveness to NE after training is that $\alpha$-adrenoceptors in vascular smooth muscle cells may be downregulated by repetitive exercise, since long-term physical activity or prolonged infusion of NE can inhibit pressor response. In 1985, Edwards et al. found that exercise training could reduce the maximal force of the aorta in the spontaneously hypertensive rats. Our results showed that the maximal contraction induced by high dose of NE was not altered by exercise training (Table 2). Although the possibility of $\alpha$-adrenoceptor desensitization in vascular smooth muscle could not be ruled out, this point of view needs to be further clarified. Our previous study demonstrated that the vascular responses to A23187, a calcium ionophore, or to sodium nitroprusside, a vasodilator directly acting on vascular smooth muscle cells, were unchanged by exercise training. In all, it appears that the training effect is more pronounced in endothelium than in smooth muscle.

To see if the alteration in vascular responses after exercise training also occurred when other vasoactive agents were used, the dose-response curves of phenylephrine-induced vasoconstriction were studied in this report as well. Although the data did not show a statistically significant difference in this experiment, there was a tendency to lower vascular tension generated by a low concentration of phenylephrine (Fig 6).

From the present results and our previous findings, we observed that exercise training could reduce NE-induced vasoconstrictive response and enhance acetylcholine-induced vasorelaxation in vitro. These effects might be the possible underlying mechanisms, at least in
part, to explain why regular exercise could protect us from some cardiovascular diseases.

In conclusion, we found that 8 weeks of endurance exercise training in normal rabbits may decrease NE-induced vasoconstrictive response in isolated thoracic aortae and that NE-stimulated EDRF release via endothelial α-receptors may, at least in part, be involved.

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References

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