Abstract—The effects of long-term aerobic exercise on endothelial function in patients with essential hypertension remain unclear. To determine whether endothelial function relating to forearm hemodynamics in these patients differs from normotensive subjects and whether endothelial function can be modified by continued physical exercise, we randomized patients with essential hypertension into a group that engaged in 30 minutes of brisk walking 5 to 7 times weekly for 12 weeks (n=20) or a group that underwent no activity modifications (control group, n=7). Forearm blood flow was measured using strain-gauge plethysmography during reactive hyperemia to test for endothelium-dependent vasodilation and after sublingual nitroglycerin administration to test endothelium-independent vasodilation. Forearm blood flow in hypertensive patients during reactive hyperemia was significantly less than that in normotensive subjects (n=17). Increases in forearm blood flow after nitroglycerin were similar between hypertensive and normotensive subjects. Exercise lowered mean blood pressure from 115.7±5.3 to 110.2±5.1 mm Hg (P<0.01) and forearm vascular resistance from 25.6±3.2 to 23.2±2.8 mm Hg/mL per minute per 100 mL tissue (P<0.01); no change occurred in controls. Basal forearm blood flow, body weight, and heart rate did not differ with exercise. After 12 weeks of exercise, maximal forearm blood flow response during reactive hyperemia increased significantly, from 38.4±4.6 to 47.1±4.9 mL/min per 100 mL tissue (P<0.05); this increase was not seen in controls. Changes in forearm blood flow after sublingual nitroglycerin administration were similar before and after 12 weeks of exercise. Intra-arterial infusion of the nitric oxide synthase inhibitor N\textsuperscript{G}-monomethyl-L-arginine abolished the enhancement of reactive hyperemia induced by 12 weeks of exercise. These findings suggest that through increased release of nitric oxide, continued physical exercise alleviates impairment of reactive hyperemia in patients with essential hypertension. (Hypertension. 1999;33[part II]:591-597.)

Key Words: exercise, aerobic ■ hyperemia, reactive ■ nitroglycerin ■ nitric oxide ■ N\textsuperscript{G}-monomethyl-L-arginine ■ endothelium ■ hypertension, essential

Regular aerobic exercise by patients with essential hypertension is associated with beneficial changes in blood pressure, lipoprotein profile, glucose metabolism, and neurohormonal release. Recent epidemiological studies have shown that physical exercise reduces cardiovascular morbidity and mortality in the general population, including individuals with hypertension.\textsuperscript{1,2} However, the mechanism underlying antihypertensive and antiatherogenic effects of exercise remains unclear.

Several lines of evidence indicate impairment of endothelium-dependent vasorelaxation in the vessels of the forearm\textsuperscript{3,4} and in the coronary\textsuperscript{5} and renal\textsuperscript{6,7} circulation of patients with essential hypertension. Recent experiments have demonstrated that continued exercise augmented vasodilation evoked by the endothelium-dependent vasodilator acetylcholine in dogs\textsuperscript{8} and rats,\textsuperscript{9} whereas in clinical studies, physical training enhanced endothelium-dependent vasodilation in the forearm in healthy subjects\textsuperscript{10} and patients with chronic heart failure.\textsuperscript{11} Whether impaired endothelium-dependent vasodilation is restored by aerobic exercise in patients with essential hypertension is, therefore, an important issue.

First, to determine whether endothelial dysfunction is demonstrable in the forearm circulation of patients with essential hypertension, we measured responses of forearm blood flow (FBF) to reactive hyperemia, an index of endothelium-dependent vasodilation, and sublingually administered nitroglycerin, an index of endothelium-independent vasodilation. Second, we evaluated effects of long-term regular aerobic exercise on endothelial function in patients with mild to moderate essential hypertension. To do this, we

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determined endothelium-dependent vasodilation at the beginning and end of a 12-week period of regular exercise.

Methods

Study Protocol 1: Endothelial Function in Normotensive and Hypertensive Subjects

We studied 27 Japanese patients with mild to moderate essential hypertension (20 men and 7 women; mean age 52.6 years). Hypertension was defined as a systolic blood pressure >160 mm Hg and/or a diastolic blood pressure >95 mm Hg measured in a sitting position on at least 3 different occasions in the outpatient clinic of Hiroshima University School of Medicine. Mild to moderate essential hypertension was defined as a systolic pressure between 140 and 170 mm Hg and a diastolic pressure between 90 and 110 mm Hg, with no objective signs of hypertensive end-organ disease. Patients with secondary forms of hypertension were excluded on the basis of a complete history and physical examination, radiologic and ultrasound examinations, and urinalysis. Plasma renin activity and plasma aldosterone and norepinephrine concentrations were determined, as well as 24-hour urinary excretion of 17-hydroxycorticosteroids, 17-ketogenic steroids, and vanillylmandelic acid. No patient had a history of cardiovascular or cerebrovascular disease, diabetes mellitus, hypercholesterolemia, liver disease, or renal disease. All patients were essentially sedentary and did not exercise regularly. Normal blood pressure was defined as a systolic blood pressure <140 mm Hg and a diastolic blood pressure <80 mm Hg. The normotensive control subjects had no history of serious disease. An alcohol intake greater than ethanol 30 mL/d also was an exclusion criterion. The study protocol was approved by the ethics committee of the First Department of Internal Medicine of Hiroshima University. Informed consent for participation was obtained from all subjects.

Vasodilatory responses to reactive hyperemia and sublingual nitroglycerin were evaluated in normotensive control subjects and essential hypertensive patients. The study began at 8:30 AM. Subjects fasted the previous night for at least 12 hours and were kept in a supine position in a quiet, dark, air-conditioned room (constant temperature, 22°C to 25°C) throughout the study. After subjects rested for 30 minutes in the supine position, their basal FBF was measured as described below. Then, the effects of reactive hyperemia and sublingual nitroglycerin on FBF were measured. To obtain flow-mediated vasodilation, FBF was occluded by inflating a cuff over the left upper arm to a pressure of 280 mm Hg for 5 minutes. After release of ischemic cuff occlusion, FBF was measured for 3 minutes. A nitroglycerin tablet (0.3 mg, Nihonkayaku Co) was administered sublingually, and again FBF was measured for 3 minutes. These studies were carried out in random order, proceeding after FBF had returned to baseline; because in a preliminary study FBF returned to baseline within 10 minutes after release of cuff occlusion or the sublingual administration of nitroglycerin, the response to reactive hyperemia or sublingual nitroglycerin was followed by a 15-minute recovery period. Baseline fasting serum concentrations of total cholesterol, LDL cholesterol, TG, creatinine, insulin, glucose, electrolytes, plasma renin activity, and norepinephrine concentration were obtained after a 30-minute rest period.

Measurement of Forearm Blood Flow

FBF was measured by using a mercury-filled Silastic strain-gauge plethysmograph (EC-5R, D.E. Hokanson, Inc) as previously described. Briefly, the strain gauge was secured to the upper part of the left arm, connected to the plethysmography device, and supported above the level of the right atrium. One minute before each measurement and throughout measurement of FBF, a wrist cuff was inflated to a pressure of 50 mm Hg greater than the systolic blood pressure to exclude the hand circulation from the measurements. The upper arm cuff was inflated to 40 mm Hg for 7 seconds in each 15-second cycle to occlude venous outflow from the arm, using a rapid cuff inflator (EC-20, D.E. Hokanson, Inc). The FBF output signal was transmitted to a recorder (U-228, Advance Co). FBF was expressed as milliliters per minute per 100 mL of forearm tissue volume. Four plethysmographic measurements were averaged to obtain FBF at baseline and during reactive hyperemia and administration of sublingual nitroglycerin.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n=17)</th>
<th>Hypertensive (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>64.2±10.3</td>
<td>62.7±11.8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.6±1.7</td>
<td>24.1±1.8</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>115.8±9.0</td>
<td>155.1±6.9*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>68.8±7.1</td>
<td>96.4±4.8*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>69.1±6.1</td>
<td>72.4±8.6</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.02±0.71</td>
<td>5.15±0.63</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.98±0.48</td>
<td>1.16±0.28</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.44±0.28</td>
<td>1.27±0.24</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.38±0.51</td>
<td>3.63±0.75</td>
</tr>
<tr>
<td>Serum glucose, mmol/L</td>
<td>4.8±0.4</td>
<td>5.0±0.4</td>
</tr>
<tr>
<td>Serum insulin, pmol/L</td>
<td>56.3±12.8</td>
<td>61.0±16.1</td>
</tr>
<tr>
<td>PRA, ng/L per second</td>
<td>0.32±0.09</td>
<td>0.28±0.11</td>
</tr>
<tr>
<td>Plasma norepinephrine, pmol/L</td>
<td>2.56±0.87</td>
<td>2.70±0.76</td>
</tr>
<tr>
<td>FBF, mL/min per 100 mL tissue</td>
<td>4.6±1.3</td>
<td>4.5±1.2</td>
</tr>
<tr>
<td>FVR, mm Hg/mL per minute per 100 mL tissue</td>
<td>18.4±4.1</td>
<td>25.7±3.2*</td>
</tr>
<tr>
<td>Smoker, No. per group</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

All results are presented as mean±SD. PRA indicates plasma renin activity; other abbreviations are defined in the text. *P<0.05 versus normotensive subjects.
Study Protocol 2. Effect of Aerobic Exercise on Endothelial Function in Patients With Essential Hypertension

Among 27 patients, 20 (14 men and 6 women; mean age 53±10 years) were randomized to regular aerobic exercise. A 4-week run-in period was followed by a 12-week physical exercise period. The other 7 patients (6 men and 1 woman; mean age 51±8 years) continued 12 weeks of follow-up with no lifestyle modification.

During the run-in period, blood pressures were stable. Vasodilatory responses to reactive hyperemia and sublingual nitroglycerin were evaluated in a protocol identical to the study protocol 1 at the beginning and end of the 12-week follow-up period.

To examine the effect of exercise on release of nitric oxide (NO), we measured FBF during reactive hyperemia and sublingual administration of nitroglycerin in the presence of the NO synthase inhibitor Nω-monomethyl-L-arginine (L-NMMA; Sigma Chemical Co) in 9 of 20 hypertensive patients randomized to aerobic exercise. The responses of forearm vasculature to reactive hyperemia and sublingual nitroglycerin after the infusion of L-NMMA were evaluated at the beginning and end of the 12-week exercise period. A 23-gauge polyethylene catheter (Hakkow Co) was inserted into the left brachial artery under local anesthesia (1% lidocaine) for infusion of L-NMMA. After the subject maintained the supine position for 30 minutes, we measured basal FBF. Then, the effects of reactive hyperemia and sublingual nitroglycerin on forearm hemodynamics were measured. FBF was measured during the last 2 minutes of the infusion. Each study was initiated after FBF had returned to baseline. After a 15-minute recovery period was completed, L-NMMA was infused intra-arterially at a dose of 8 μmol/min for 5 minutes for basal FBF measurement. We performed reactive hyperemia and sublingual nitroglycerin after initiation of a 5-minute infusion of L-NMMA; FBF was measured during the last 2 minutes of infusion.

Aerobic Exercise

Subjects performed 30 minutes of brisk walking 5 to 7 times per week for 12 weeks. A 5-minute warm-up period was followed by 30 minutes of exercise and a 5-minute cool-down period. We explained the method of aerobic exercise in detail (exercise type, frequency, duration, and intensity) and demonstrated brisk walking for the subjects. Participants were asked to record exercise performed but otherwise maintain their original behavioral and dietary habits, especially their intake of sodium, potassium, calories, and alcohol.

To monitor compliance, we checked the exercise performance sheet, measured 24-hour urinary excretion of sodium and potassium, and interviewed all subjects every 4 weeks. In a preliminary study, the intensity of brisk walking ordered was equivalent to 52±6% of maximum oxygen consumption (n=5).

Analytical Methods

Samples of venous blood were placed in tubes containing sodium EDTA (1 mg/mL) and in polystyrene tubes. The EDTA-containing tubes were chilled promptly in an ice bath preceding immediate separation of plasma by centrifugation at 3100 rpm at 4°C for 10 minutes; serum was separated at 1000 rpm at room temperature for 10 minutes. Samples were stored at −80°C until assayed. Routine clinical methods were used to determine serum concentrations of total cholesterol, HDL cholesterol, TG, creatinine, glucose, and electrolytes. Serum concentration of low-density lipoprotein (LDL) was determined using Friedewald’s method.12 PRA (Gamma Coat PRA, Baxter Travenol Co) was assayed by radioimmunoassay. The plasma concentration of norepinephrine was measured by high-performance liquid chromatography.

Statistical Analysis

Results are presented as the mean±SD. Values of P<0.05 were considered significant. The Mann-Whitney U test was used to evaluate differences between hypertensive subjects and normotensive subjects concerning parameters at baseline. Comparisons of parameters before and after exercise were performed with adjusted means by ANCOVA using baseline data as covariates. Comparisons of time course curves of parameters during reactive hyperemia were analyzed by 2-way ANOVA for repeated measures. The data were processed using the software packages StatView IV (Brainpower) or Super ANOVA (Abacus Concepts).

Results

Study Protocol 1. Effects of Reactive Hyperemia and Sublingual Nitroglycerin on Forearm Blood Flow in Patients With Essential Hypertension and Normotensive Subjects

Baseline clinical characteristics in the 27 hypertensive patients before exercise and the 17 normotensive control subjects are summarized in Table 1. Systolic and diastolic blood pressures as well as forearm vascular resistance (FVR) were significantly higher in hypertensive patients than in normotensive subjects. Other parameters were similar in the 2 groups. The FBF of the hypertensive patients during reactive hyperemia, an index of endothelium-dependent vasorelaxation, was significantly less than in normotensive subjects (Figure 1A), whereas the increase in FBF after sublingually administered nitroglycerin, an
index of endothelium-independent vasodilation, was similar in the 2 groups (Figure 1B).

**Study Protocol 2. Effects of Aerobic Exercise on Baseline Clinical Characteristics**
The baseline clinical characteristics in the 20 hypertensive patients undergoing the aerobic exercise program (exercise group) and the 7 hypertensive patients with no change in activities (control group) are summarized in Table 2, both before and after the 12-week period. The baseline values for all parameters were similar in the 2 groups. A 12-week period of exercise significantly decreased systolic and diastolic arterial blood pressures, the concentration of LDL cholesterol, and FVR, and increased significantly the concentration of HDL cholesterol, while no changes occurred in the control group. Other parameters, such as basal FBF and blood glucose, remained unchanged after 12 weeks in both groups.

**Effects of Aerobic Exercise on Endothelial Function**
At the baseline measurement, reactive hyperemia was similar in the exercise and control groups. After 12 weeks of exercise, the maximal FBF response in reactive hyperemia increased significantly in the exercise group, from 38.4 ± 4.6 to 47.1 ± 4.9 mL/min per 100 mL tissue (P < 0.05) (Figure 2A), but no change was detected in the control group. Changes in FBF after sublingual nitroglycerin administration were similar before and after the 12-week interval in both groups (Figure 2B).

The increase in maximal FBF with reactive hyperemia after 12 weeks of exercise significantly correlated with the decrease in LDL cholesterol in the exercise group (r = −0.46, P < 0.05); (Figure 3). No significant correlation was seen between maximal FB with reactive hyperemia and changes in blood pressure or other parameters.

**Effects of L-NMMA on the Forearm Vascular Response to Reactive Hyperemia and Nitroglycerin Before and After the 12-Week Exercise Period**
The FBF response was evaluated both in the absence and presence of L-NMMA. In 9 of 20 hypertensive patients performing aerobic exercise, reactive hyperemia had increased significantly after 12 weeks (Figure 4A). Intravenous infusion of the NO synthase inhibitor L-NMMA significantly decreased basal FBF, from 4.8 ± 1.1 to 3.0 ± 0.6 mL/min per 100 mL tissue (P < 0.05). The change in basal forearm vascular responses to L-NMMA was similar at the 0- and 12-week time points. No significant changes in arterial blood pressure or heart rate were detected during infusion of L-NMMA at 0 and 12 weeks.

Intra-arterial infusion of L-NMMA decreased the response to reactive hyperemia at both time points and abolished enhancement of reactive hyperemia induced by 12 weeks of exercise (Figure 4B).
ethanol), sodium intake, potassium intake, and cigarette smoking were similar in both exercise and control groups throughout the study, suggesting that increased regular physical activity enhanced endothelium-dependent vasodilation, reduced blood pressure, and improved the lipoprotein profile.

Long-term aerobic exercise in hypertensive patients improved reactive hyperemia, an index of endothelium-dependent vasorelaxation, through an increase in release of NO. Nitroglycerin-induced vasodilation did not change over 12 weeks in exercise or control groups. Results are presented as mean±SEM. The probability value refers to a comparison of time-course curves using ANOVA for repeated measurements.

Figure 2. Forearm blood flow at rest and during reactive hyperemia (A), and after the sublingual administration of nitroglycerin (B). Before exercise, reactive hyperemia was similar in the exercise group (n=20) and the hypertensive control group (n=7). Twelve weeks of aerobic exercise improved reactive hyperemia. Nitroglycerin-induced vasodilation did not change over 12 weeks in exercise or control groups. Results are presented as mean±SEM. The probability value refers to a comparison of time-course curves using ANOVA for repeated measurements.

Figure 3. Scatterplots show the relationship between the increase in maximal forearm blood flow response to reactive hyperemia and the change in LDL cholesterol after 12 weeks of exercise in the exercise group (n=20). The increase in maximal forearm blood flow response to reactive hyperemia correlated significantly with the change in LDL cholesterol.

Figure 4. Forearm blood flow at rest and during reactive hyperemia in 8 of 20 hypertensive patients receiving a nitric oxide synthase inhibitor L-NMMA before and after 12 weeks of aerobic exercise. Without L-NMMA, reactive hyperemia increased significantly (A). Intra-arterial infusion of L-NMMA decreased the response to reactive hyperemia at both time points and abolished the enhanced reactive hyperemia associated with 12 weeks of exercise (B).
These findings suggest that endothelial dysfunction is important in the increased vascular resistance observed in hypertensive patients.

Long-term aerobic exercise improved endothelium-dependent vasodilation with reactive hyperemia but not endothelium-independent vasodilation in response to nitroglycerin. These findings suggest that exercise restored normal function mainly in vascular endothelium, not vascular smooth muscle. Although the present study did not determine the mechanism by which improved endothelial function resulted from regular aerobic exercise, some possibilities might be considered. First, aerobic exercise increases shear stress, which triggers release of NO. Repetitive increases in blood flow or shear stress with exercise may enhance NO release in the vascular endothelium; indeed, physiological levels of shear stress can induce NO release from cultured endothelial cells. Sessa et al recently demonstrated that increasing endothelial shear stress by continued exercise may increase NO production and levels of coding for mRNA NO synthase, which may contribute to beneficial effects of exercise in the epicardial coronary arteries of dogs. Increased blood flow and shear stress also have been shown to have beneficial effects on vascular structure and reactivity.

A second possible mechanism is based on observations that oxidized LDL interferes with formation of NO and even directly inactivates NO. Several lines of evidence have shown that cholesterol-lowering and antioxidant therapy can restore impaired endothelium-dependent vasodilation in forearm arteries. Although we did not directly measure oxidized LDL levels in the present study, the increase in FBF response to reactive hyperemia correlated with a decrease in LDL cholesterol after 12 weeks of exercise. The reduction in LDL may result in less suppression of NO formation by oxidized LDL.

Finally, several experimental findings suggest that endothelial function is impaired in relation to the severity of blood pressure elevation. Thus, reduction of blood pressure per se may improve endothelial function. However, we found no significant correlation between reduction in blood pressure by exercise and increase in FBF response to reactive hyperemia. Previous studies also have concluded that decrease in blood pressures do not directly improve endothelial function in the brachial artery and small arteries of patients with essential hypertension. We therefore doubt this last possibility.

Reactive hyperemia is thought to have components of endothelium dependence and independence and to be multifactorial. In the present study, NO synthase inhibitor L-NMMA abolished reactive hyperemia by approximately 40% before exercise. Reactive hyperemia was reduced by adenosine receptor antagonist theophylline but not cyclooxygenase inhibitor aspirin. Endothelium-derived hyperpolarizing factor may also contribute to reactive hyperemia. Because the enhanced response of the forearm vasculature to reactive hyperemia by exercise in the exercising group was inhibited substantially by L-NMMA, we consider the augmentation of NO release to be involved in exercise-enhanced reactive hyperemia. It is reported that this endothelium-dependent vasodilation evoked by intra-arterial infusion of vasoactive agents has been substituted by vascular responses to reactive hyperemia, a noninvasive method. The use of agonists such as acetylcholine or methacholine to stimulate NO release would allow us to draw more specific conclusions concerning the role of the basal and stimulated release of NO by exercise in the forearm circulation.

In conclusion, moderately intense regular aerobic physical activity prevented impairment of reactive hyperemia in patients with essential hypertension, most likely because of an exercise-induced increase in production of NO. Aerobic exercise should be recommended to aid in prevention of hypertension, to reduce blood pressure in established hypertension, and to reserve endothelial dysfunction related to hypertension.

Acknowledgments

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Daily Aerobic Exercise Improves Reactive Hyperemia in Patients With Essential Hypertension
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