Effect of Different Intensities of Exercise on Endothelium-Dependent Vasodilation in Humans
Role of Endothelium-Dependent Nitric Oxide and Oxidative Stress

Chikara Goto, RPT, MS; Yukihito Higashi, MD, PhD; Masashi Kimura, MD; Kensuke Noma, MD; Keiko Hara, MD; Keigo Nakagawa, MD; Mitsutoshi Kawamura, RPT, MS; Kazuaki Chayama, MD, PhD; Masao Yoshizumi, MD, PhD; Isao Nara, RPT, PhD

Background—Aerobic exercise enhances endothelium-dependent vasodilation in hypertensive patients, patients with chronic heart failure, and healthy individuals. However, it is unclear how the intensity of exercise affects endothelial function in humans. The purpose of the present study was to determine the effects of different intensities of exercise on endothelium-dependent vasodilation in humans.

Methods and Results—We evaluated the forearm blood flow responses to acetylcholine, an endothelium-dependent vasodilator, and isosorbide dinitrate, an endothelium-independent vasodilator, before and after different intensities of exercise (mild, 25% VO$_2$max; moderate, 50% VO$_2$max; and high, 75% VO$_2$max; bicycle ergometers, 30 minutes, 5 to 7 times per week for 12 weeks) in 26 healthy young men. Forearm blood flow was measured using a mercury-filled Silastic strain-gauge plethysmograph. Twelve weeks of moderate-intensity exercise, but not mild- or high-intensity exercise, significantly augmented acetylcholine-induced vasodilation (7.5±2.4 to 11.4±5.8 mL/min per 100 mL tissue; P<0.05). No intensity of aerobic exercise altered isosorbide dinitrate–induced vasodilation. The administration of NOS-monomethyl-L-arginine, a nitric oxide synthase inhibitor, abolished the moderate-intensity exercise-induced augmentation of the forearm blood flow response to acetylcholine. High-intensity exercise increases plasma concentrations of 8-hydroxy-2’-deoxyguanosine (from 6.7±1.1 to 9.2±2.3 ng/mL; P<0.05) and serum concentrations of malondialdehyde-modified low-density lipoprotein (from 69.0±19.5 to 82.4±21.5 U/L; P<0.05), whereas moderate exercise tended to decrease both indices of oxidative stress.

Conclusions—These findings suggest that moderate-intensity aerobic exercise augments endothelium-dependent vasodilation in humans through the increased production of nitric oxide and that high-intensity exercise possibly increases oxidative stress. (Circulation. 2003;108:530-535.)

Key Words: endothelium ■ exercise ■ nitric oxide ■ free radicals ■ blood flow

Recent epidemiological studies have shown that physical exercise reduces cardiovascular morbidity and mortality in the general population, including healthy subjects.1,2 Recent experimental studies demonstrated that continued exercise augments the vasodilation evoked by the endothelium-dependent vasodilator acetylcholine (ACh) in dogs3 and rats.4 In human studies, physical training enhanced endothelium-dependent vasodilation in forearm circulation, hypertensive patients, patients with chronic heart failure,5 and healthy individuals.6 It is clinically important to select the appropriate intensity, duration, frequency, and kind of exercise, because intense exercise can be hazardous to human vessels. Exercise intensity is quite important. However, it is unclear how the intensity of exercise affects endothelial function in humans.

To determine the role of different intensities of exercise on endothelial function, we measured the responses of forearm blood flow (FBF) to the endothelium-dependent vasodilator ACh and the endothelium-independent vasodilator isosorbide dinitrate (ISDN) before and after 12 weeks of exercise.

Methods

Subjects
We studied 26 healthy Japanese men (mean age, 25±2.5 years). After measuring maximum oxygen consumption (VO$_2$max) to decide on the intensity of exercise, subjects were divided into three groups (mild intensity, 25% VO$_2$max, n=10; moderate intensity, 50% VO$_2$max, n=8; and high intensity, 75% VO$_2$max, n=8). All subjects did not have an exercise habit. They had no history of serious disease and took no medications for at least 4 weeks before the study. The

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From the Program in Physical Therapy Health Sciences, School of Medicine (C.G., M.K., I.N.), Department of Cardiovascular Physiology and Medicine (Y.H., M.Y.), Department of Medicine and Molecular Science (K. Noma, M.K., K. Nakagawa, K.C.), Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan.

Correspondence to Yukihito Higashi, MD, PhD, FAHA, Department of Cardiovascular Physiology and Medicine, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. E-mail yhigashi@hiroshima-u.ac.jp

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study protocol was approved by the ethics committees of the Hiroshima University Faculty of Medicine. Informed consent for participation was obtained from all subjects.

Exercise

All subjects performed 30 minutes of bicycle ergometer 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 exercise in detail (exercise type, frequency, duration, and intensity) and demonstrated the bicycle ergometer for the subjects. Participants were asked to record the exercise performed but to 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, we confirmed the effects the intensities of exercise (25%, 50%, and 75% VO\textsubscript{max}) had on movement intensities after 12 weeks in the same subjects in whom endothelial function had been evaluated. Twelve weeks of each exercise regimen (25%, 50%, and 75% VO\textsubscript{max}) significantly increased VO\textsubscript{max} by 7±4%, 13±9%, and 12±10%, respectively (all P<0.01). There was a significant difference in exercise intensities for VO\textsubscript{max} after 25% VO\textsubscript{max} exercise, 50% VO\textsubscript{max} before exercise, VO\textsubscript{max} after 50% VO\textsubscript{max} exercise, 75% VO\textsubscript{max} before exercise, and VO\textsubscript{max} after 75% VO\textsubscript{max} exercise (respectively, P<0.01). Although VO\textsubscript{max} significantly increased after each exercise, exercise intensity was limited to mild, moderate, and high levels, even after exercise training.

Measurement of FBF

The FBF was measured with a mercury-filled Silastic strain-gauge plethysmograph (EC-5R, DE Hokanson, Inc), as previously described. Briefly, a strain gauge was attached to the upper part of the left arm, connected to a plethysmography device, and supported above the right atrium. A wrist cuff was inflated to 50 mm Hg above the systolic blood pressure to exclude hand circulation from the measurements taken 1 minute before measurement of FBF. The upper arm—congesting cuff was inflated to 40 mm Hg for 7 s at a 15-s cycle to occlude venous outflow from the arm by using a rapid cuff inflator (EC-20, DE Hokanson, Inc). The FBF output signal was transmitted to a recorder (U-228, Advance Co). FBF is expressed as milliliters per minute per 100 milliliters of forearm tissue volume. Forearm vascular resistance was calculated as the mean blood pressure divided by FBF and is expressed as mm Hg per milliliter per minute per 100 milliliters of forearm tissue volume. Four plethysmographic measurements were averaged to obtain the FBF at baseline and during the infusion of ACh and ISDN. FBF was calculated by 2 independent observers who were blinded to the study protocol from the linear portions of plethysmographic recordings. The intraobserver coefficient of variation was 3.0%. We confirmed the reproducibility of FBF responses to ACh and ISDN on 2 separate occasions in 10 healthy men (mean age, 24±4 years). The coefficients of variation were 6.2% and 4.6%, respectively.

Measurements of FBF were performed before exercise and after 12 weeks of exercise. The FBF measurement study began at 8:30 AM. Subjects fasted the previous night for at least 12 hours. They were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature, 22°C to 25°C) throughout the measurement study. Subjects remained in a supine position for 30 minutes, and then basal FBF was measured. Then, the effects of an infusion of ACh and ISDN on FBF were measured. The ACh infusion was administered at doses of 3.75 and 7.5 µg/min, and the ISDN infusion was administered at doses of 1.5 and 3.0 µg/min. After the administration of ACh and ISDN, FBF was measured during the last 2 minutes of the infusion. These studies were performed in a randomized fashion. Each study proceeded after FBF had returned to baseline. In the preliminary study, after the infusion of ACh or ISDN, FBF returned to baseline within 30 minutes. Thus, the end of the infusion of ACh or ISDN was followed by a 30-minute recovery period. Baseline fasting serum concentrations of total cholesterol, HDL cholesterol, triglycerides, creatinine, insulin, glucose, electrolytes, and ACE activity, plasma renin activity (PRA), and norepinephrine concentrations were obtained after a 30-minute rest period.

To examine the effect of exercise on the release of nitric oxide (NO), we measured FBF during the infusion of ACh and ISDN in the presence of the NO synthase inhibitor N\textsuperscript{\textcircled{\textscriptsize{6}}} monomethyl-L-arginine.

| Baseline Clinical Characteristics of Mild- (25% VO\textsubscript{max}), Moderate- (50% VO\textsubscript{max}), and High-Intensity (75% VO\textsubscript{max}) Exercise |
|---------------------------------|----------------|----------------|----------------|
| Variables                       | Before         | After          | Before         | After          | Before         | After          |
| Body weight, kg                 | 652±3.6        | 643.4±4.3      | 656.6±6.5      | 633.3±5.8      | 658.0±4.1      | 659.9±4.3      |
| Systolic blood pressure, mm Hg  | 115.4±9.0      | 114.5±8.9      | 121.4±12.0     | 120.9±11.2     | 116.8±5.0      | 115.3±9.9      |
| Diastolic blood pressure, mm Hg | 61.3±4.6       | 64.8±8.3       | 63.0±6.0       | 63.2±5.8       | 60.8±4.4       | 61.1±5.4       |
| Heart rate, bpm                 | 59.6±7.9       | 60.0±5.9       | 61.0±12.4      | 57.5±10.2      | 60.3±8.0       | 61.0±6.3       |
| Serum insulin, pmol/L           | 30.5±26.4      | 45.6±10.8      | 36.0±19.3      | 28.8±12.9      | 34.8±18.6      | 39.0±21.6      |
| HDL cholesterol, mmol/L         | 1.4±0.3        | 1.5±0.2        | 1.3±0.4        | 1.3±0.4        | 1.4±0.3        | 1.5±0.3        |
| Serum glucose, mmol/L           | 5.3±0.9        | 4.7±0.4        | 5.3±0.9        | 4.8±0.5        | 5.4±1.2        | 4.7±0.4        |
| Triglycerides, mmol/L           | 0.8±0.6        | 0.8±0.4        | 0.9±0.6        | 1.0±0.8        | 0.6±0.2        | 0.7±0.3        |
| Total cholesterol, mmol/L       | 4.1±0.7        | 4.2±0.8        | 4.6±0.8        | 4.8±1.0        | 3.9±0.6        | 3.9±0.5        |
| Plasma norepinephrine, pg/mL    | 1022.6±222.3   | 952.9±284.3    | 1182.8±331.6   | 1158.0±258.9   | 1101.9±272.5   | 1172.2±433.3   |
| Renin-angiotensin system         |                |                |                |                |                |                |
| Plasma renin activity, ng/mL per hour | 2.3±1.1   | 2.3±2.0        | 1.9±1.2        | 2.0±1.0        | 2.2±1.1        | 3.1±1.6        |
| Plasma angiotensin II, pg/mL    | 6.6±2.7        | 8.6±4.5        | 6.8±3.1        | 5.8±1.9        | 8.7±4.7        | 10.4±3.1       |
| Plasma aldosterone, pg/mL       | 69.5±11.9      | 79.2±20.6      | 67.5±14.9      | 106.0±24.6     | 70.0±10.4      | 84.5±22.2      |
| Forearm hemodynamics             |                |                |                |                |                |                |
| FBF, mL/min per 100 mL tissue   | 5.0±1.4        | 4.8±1.0        | 5.1±1.0        | 5.3±1.7        | 5.2±1.9        | 4.5±1.1        |
| FVR, mm Hg/mL per min per 100 mL tissue | 16.9±4.3 | 16.9±3.6     | 16.8±3.5       | 16.9±5.8       | 17.3±6.9       | 18.9±5.6       |

Smoker, No. per group            | 5              | 4              | 4              |

All results are mean±SD. FBF indicates forearm blood flow; FVR, forearm vascular resistance.
(L-NMMA; Sigma) in all subjects. The responses of forearm vasculature to ACh and ISDN after the infusion of L-NMMA were evaluated at the beginning and end of the 12-week exercise period.

**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 chemical methods were used to determine serum concentrations of total cholesterol, HDL cholesterol, triglycerides, creatinine, glucose, and electrolytes. Serum concentrations of LDL cholesterol were determined using Friedewald's method. Plasma renin activity (Gamma Coat PRA, Baxter Travenol Co) was assayed by radioimmunoassay. The plasma concentration of norepinephrine was measured by high-performance liquid chromatography. The plasma concentration of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was assayed by an enzyme-linked immunosorbent assay (ELISA) using 8-OHdG kits (Nihon Yushi Co). The serum concentration of malondialdehyde-modified LDL (MDA-LDL) was also assayed by ELISA (anti-MDA-LDL antibody, SRL Co).

**Statistical Analysis**

Results are presented as the mean±SD. P<0.05 was considered significant. The Mann-Whitney U test was used to evaluate differences in subjects between before and after exercise with regard to 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 curves of parameters infusing ACh or ISDN were analyzed by 2-way ANOVA for repeated measures. The data were processed using the software packages Stat View IV (Brainpower) or Super ANOVA (Abacus Concepts).

**Results**

**Clinical Characteristics**

Baseline clinical characteristics in all subjects are summarized in the Table. There was no significant difference among the 3 groups before exercise. Intensity of exercise did not alter systemic hemodynamics, lipid profiles, or glucose metabolism.

**Effect of Exercise on Endothelial Function**

The intra-arterial infusion of ACh increased FBF in a dose-dependent manner in all 3 groups. Twelve weeks of moderate exercise (50% VO2max) augmented the FBF responses to ACh (maximum, 13.1±8.8 to 19.6±12.7 mL/min per 100 mL tissue), whereas mild and high intensities of exercise did not alter the FBF responses to ACh (Figure 1). The intra-arterial infusion of ISDN increased FBF in a dose-dependent manner in all 3 groups. FBF responses to ISDN were similar before and after exercise in all 3 groups (Figure 2). An intra-arterial infusion of the NO synthase inhibitor L-NMMA significantly decreased basal FBF and FBF responses to ACh in all 3 groups. In the moderate-intensity exercise group, L-NMMA abolished the augmentation of FBF responses to ACh after 12 weeks of exercise (Figure 3). No significant changes in arterial blood pressure or heart rate were detected during the infusion of L-NMMA in any group.

**Effect of Exercise on Oxidative Stress**

Twelve weeks of high-intensity exercise increased plasma 8-OHdG levels from 6.7±1.1 to 9.2±2.3 ng/mL (P<0.05) and serum MDA-LDL levels from 69.0±19.5 to 82.4±21.5 U/L (P<0.05). Although mild and moderate exercise did not significantly alter plasma 8-OHdG and serum MDA-LDL levels, moderate exercise had a tendency to decrease both parameters (8-OHdG, 8.2±2.7 to 7.1±1.8 ng/mL; MDA-LDL, 68.4±14.3 to 61.7±12.7 U/L; Figures 4 and 5).

**Discussion**

In the present study, 12 weeks of moderate exercise, but not mild- or high-intensity exercise, augmented endothelium-dependent vasodilation through increased NO production in healthy subjects. High-intensity exercise increased plasma concentrations of 8-OHdG and serum concentrations of MDA-LDL, which are indices of oxidative stress.

**Figure 1.** The effects of mild-, moderate-, and high-intensity exercise on FBF responses to ACh.

**Figure 2.** The effects of mild-, moderate-, and high-intensity exercise on FBF responses to ISDN.
Relationship Between Exercise Intensity and Endothelial Function

Moderate-Intensity Exercise

A 12-week moderate-intensity exercise program improved endothelium-dependent vasodilation with ACh, but not endothelium-independent vasodilation with ISDN. These findings indicate that the augmentation of ACh-induced vasorelaxation may be related to an improvement in the function of endothelium, but not vascular smooth muscle. There are several possible explanations for the augmented forearm vascular response to ACh by regular aerobic exercise in humans. Several lines of evidence have shown that regular physical exercise is associated with beneficial changes in blood pressure, lipid metabolism, glucose metabolism, neurohormonal factors, body weight, and shear stress.2,10–14 Although it has been postulated that the decrease in blood pressure and vasoconstricting agents, altered lipoprotein profile, and increase in shear stress may contribute to endothelium-dependent vasodilation, the precise mechanism by which regular aerobic exercise training improves endothelial dysfunction remains unknown. Several investigators have reported the possibility that acute and chronic exercise-induced endothelium-dependent vasodilation is due mainly to an increase in NO release.1,3–5,15 In the present study, to determine the role of NO in ACh-induced forearm vasodilation after 12 weeks of exercise, we studied the effects of the NO synthase inhibitor L-NMMA. We showed that the enhanced response of the forearm vasculature to ACh by exercise in the exercising group was substantially inhibited by L-NMMA, thus suggesting that the augmentation of NO release is involved in exercise-enhanced endothelium-dependent vasorelaxation.

One possible mechanism by which long-term aerobic exercise augments ACh-stimulated NO release is an increase in vascular shear stress resulting from increased flow. Acute or chronic increases in shear stress patently stimulate the release of NO in isolated vessels16 and cultured cells.17 Recently, Sessa et al18 demonstrated that, in the epicardial coronary arteries of dogs, the increase in shear stress from 10 days of treadmill exercise enhanced the expression of the vascular endothelial constitutive NO synthase gene, leading to ACh-stimulated NO release. In addition, chronic increases in shear stress have been shown to lead to functional and histological alterations of vascular endothelium, resulting in enhanced vascular structure and function.19 This beneficial change in endothelium after long-term aerobic exercise may also contribute to the augmented forearm vasorelaxation to ACh and ACh-stimulated NO release.

A balance between ambient levels of superoxide and released NO plays a critical role in the maintenance of normal endothelial function. Oxidized LDL, LDL that has undergone oxidative modification, has been shown to interfere with the formation of NO20 and to inactivate NO directly.21 Tamai et al22 demonstrated that even a single LDL apheresis improved endothelium-dependent vasodilation in forearm vessels of hypercholesterolemic humans. Thirty minutes of bicycle ergometer or treadmill training 3 times weekly for 12 weeks decreased oxidative stress in patients with ischemic heart diseases.23 However, in the present study, a 12-week moderate-intensity exercise program did not significantly alter plasma 8-OHdG, one of the most commonly used markers for evaluating oxidative DNA damage, or serum MDA-LDL levels, one indicator of oxidized LDL in healthy young men.

A balance of vasodilators and vasoconstrictors also plays an important role in the physiological regulation of vascular tone.24–26 It is well known that various vasoconstricting factors released from the endothelium, such as angiotensin II, affect endothelium-dependent vasodilation in humans. We have confirmed that circulating levels of angiotensin II are similar before and after any intensity of exercise. However, we cannot deny the possibility that other vasoconstrictors contribute to differences in the degree of ACh-stimulated vasodilation.

Norepinephrine, which acts as a potent vasoconstrictor, attenuates endothelium-dependent vasodilation. However,
plasma norepinephrine concentrations were similar before and after any intensity of exercise. Therefore, the differences in FBF response to ACh before and after moderate-intensity exercise cannot be explained by differences in sympathetic nervous system activity.

**Mild- and High-Intensity Exercise**

In the present study, mild-intensity exercise did not alter any parameters, including oxidative stress or endothelial function. Interestingly, 12 weeks of high-intensity exercise increased plasma concentrations of 8-OHdG and serum concentrations of MDA-LDL in healthy young men. Davies et al. reported that the massive increase in oxygen uptake that occurs in skeletal muscle in exercise is associated with an increase in free radical formation. The 75% \( \dot{V}O_2 \max \) intensity level is above the recommended aerobic exercise level. Intense exercise should be avoided because excess exercise can be hazardous to human vessels. Bergholm et al. showed that 12 weeks of intense physical training at 70% to 80% \( \dot{V}O_2 \max \), consisting of four 1-hour running sessions per week, resulted in decreases in circulating antioxidants, such as alpha-tocopherol and beta-carotene, in healthy men. These findings suggest that long-term intense (anaerobic) exercise may impair endothelium-dependent vasodilation through decreases in levels of antioxidants and an increase in reactive oxygen species, resulting in a reduction in NO bioavailability. However, we were unable to find impaired endothelial function associated with increased oxidative stress in healthy subjects. Matsumoto et al. reported that the production of NO progressively increases as exercise intensity increases. Although we did not assess the production of NO, there is a possibility that this high-intensity exercise increases NO production. The action of increased oxidative stress that inactivates NO bioavailability was removed by increased NO production, resulting in a maintenance of endothelial function.

**Study Limitations**

It is well known that the release of prostaglandins and endothelium-derived hyperpolarizing factor may also contribute to exercise-induced vasodilation. In the present study, the augmented endothelium-dependent vasorelaxation after 12 weeks of exercise was inhibited substantially by L-NMMA; therefore, we think that an increase in NO release may be a large contributor to augmented ACh-induced vasodilation by long-term aerobic exercise. In addition, the administration of prostaglandin synthesis inhibitors reduced exercise-induced vasodilation by only \( \approx 10\% \) in humans, suggesting that prostaglandins may play a minimal role in exercise-induced vasodilation. Examination of the effects of prostaglandins and endothelium-derived hyperpolarizing factor would have allowed us to draw more specific conclusions about the role of aerobic exercise in endothelium-dependent vasodilation.

In the present study, plasma 8-OHdG levels tended to be reduced by moderate exercise, which augments ACh-induced vasodilation. Some investigators have shown that there is a significant relationship between plasma 8-OHdG levels and urinary 8-OHdG or tissue 8-OHdG levels. However, we cannot deny the possibility that measuring plasma 8-OHdG using an ELISA technique is not sufficient to draw conclusions about oxidative stress in mild to moderate exercise. Measurement of urinary 8-OHdG excretion may enable us to draw more specific conclusions about the role of oxidative stress mediated by exercise.

In conclusion, long-term moderate-intensity exercise, but not mild- or high-intensity exercise, augments endothelium-dependent vasodilation in healthy subjects. This moderate-intensity exercise fits the index of exercise training that is recommended from the preventive general viewpoint of cardiovascular diseases. At present, there are no data in the literature that would support the statement that a high-intensity exercise regimen is harmful with regard to cardiovascular diseases. In addition, it is unclear whether a long-term moderate exercise regimen per se reduces cardiac events. Prospective studies are needed to clarify the effects of different intensities of exercise on the outcome of cardiovascular diseases.

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