Original Article

Moderate Regular Exercise Increases Basal Production of Nitric Oxide in Elderly Women

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Vascular endothelial cells produce nitric oxide (NO), which is a potent vasodilator substance and is thought to have antiatherosclerotic properties. Therefore, it has also been proposed that NO may be useful to regulate vascular tonus and prevent progression of atherosclerosis. On the other hand, NO activity reduces with aging. We previously reported that the plasma nitrite/nitrate (NOx: the stable end product of NO) concentration was significantly increased by intense aerobic exercise training in healthy young humans. We hypothesized that lifestyle modification (*e.g.*, even mild regular exercise training) can increase NO production in previously sedentary older humans. We measured the plasma NOx concentration before and after a mild aerobic exercise training regimen (cycling on a leg ergometer at 80% ventilatory threshold for 30 min, 5 days/week) for 3 months in elderly women. In addition, we assessed the plasma concentration of cyclic guanosine monophosphate (cGMP), a second messenger of NO, in the same samples. The individual ventilatory threshold increased significantly after the 3-month exercise training. The blood pressure at rest significantly decreased after exercise training. These results suggest that the 3-month exercise training in the older women produced favorable physiological effects. The plasma concentration of NOx significantly increased by the exercise training, and the plasma concentration of cGMP also increased by the exercise training. The present study suggests that even a mild regular aerobic-endurance exercise increases NO production in previously sedentary older humans, which may have beneficial effects (*i.e.*, antihypertensive and antiatherosclerotic effects by endogenous NO) on the cardiovascular system. (*Hypertens Res* 2004; 27: 947−953)

***Key Words*: nitric oxide, cyclic guanosine monophosphate, aging, lifestyle, exercise training**

Introduction

It has been well demonstrated that the loss of endothelial function is not only characteristic of diseases such as essential or secondary hypertension (*1*, *2*) and atherosclerosis (*3*, *4*), but is also associated with advancing age (*5*). It has been reported that, independent of the presence of other pathological states, aging impairs vascular endothelial function (*6*–*10*). The alteration of endothelial function occurring with aging may have important clinical implications in the pathogenesis of cardiovascular disease.

Vascular endothelial cells play an important role in the regulation of vascular activity by producing vasoactive sub-

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stances. Nitric oxide (NO) produced in the vascular endothelial cells shows a potent vasodilator effect (*11*, *12*), and plays an important role in the local regulation of platelet-vessel wall interactions and in vascular resistance and growth (*11–14*). On the basis of these effects, NO has been proposed to have antihypertensive, antithrombotic, and antiatherosclerotic properties. Reduction in basal NO release may predispose to hypertension, thrombosis, vasospasm, and atherosclerosis (*15–21*). The plasma nitrite/nitrate (NOx: the stable end product of NO) concentration is decreased in some human cardiovascular diseases, *e.g.*, essential hypertension (*22*) and atrial fibrillation (*23*). On the other hand, plasma NOx concentrations in patients with noninsulin-dependent diabetes mellitus (*24*) and refractory arterial hypertension (*25*) have been reported not to be different from those of healthy subjects. Although there is a discrepancy in the results of the measurement of NOx level in plasma, the decrease in plasma NOx concentration may have important clinical significance in the pathophysiology of some diseases. Furthermore, it has been reported that a decrease in NO bioavailability may occur with aging (*6*, *26–28*). On the other hand, we previously showed that the plasma NOx concentration was significantly increased by intense aerobic exercise training in healthy young humans (*29*). It has also been reported that intense aerobic exercise training is associated with improved NO bioavailability in elderly athletes (*30*). These study have been observed improvement of NO system by high-intensity exercise training. However, the efficacy of low-intensity exercise training for increasing NO production has not been established. We considered that it would be of great interest to study whether mild (low-intensity) exercise training would cause an increase in NO production in previously sedentary older humans.

Accordingly, the purpose of the present study was to examine whether NO production is increased by lifestyle modification (*e.g.*, mild regular exercise training) in previously sedentary older humans. We measured the plasma NOx concentration before and after mild aerobic exercise training (cycling on a leg ergometer at 80% ventilatory threshold for 30 min, 5 days/week) for 3 months in elderly women. To further confirm the results, the level of cyclic guanosine monophosphate (cGMP), a second messenger of NO, in the plasma was also measured before and after the exercise training.

Methods

Subjects

Fifteen untrained older women (59–69 years old) volunteered to participate. All subjects were normotensive (<140/90 mmHg), nonobese, and free from the signs, symptoms and history of any overt chronic diseases. None of the participants had a history of smoking or hormone replacement therapy, and none were currently taking any medications. The subjects were randomly divided into two groups. One group completed a course of exercise intervention (exercise group: $n=10$; mean age, 63 ± 4 years) and the other group served as a sedentary control group ($n=5$; mean age, 64 ± 4 years).

The study was approved by the Ethical Committee of the Institute of Health and Sport Sciences, the University of Tsukuba. This study conformed with the principles outlined in the Declaration of Helsinki, and all subjects gave their written informed consent before inclusion in the study.

Experimental Design

The exercise group was submitted to a 3-month exercise training program on a cycle ergometer for 30 min/day, 5 days/week at 80% of their individual ventilatory threshold (VT). Individual VT, resting systolic blood pressure (SBP), resting diastolic blood pressure (DBP), resting heart rate, resting venous plasma NOx concentration, and resting venous plasma cGMP concentration were measured before and after the aerobic exercise training program or the sedentary program. Serum concentrations of cholesterol, triglyceride, and insulin and plasma glucose concentration were also measured before and after the programs. In addition, an index of relative insulin resistance was evaluated using homeostasis model assessment (HOMA) (*31*). Before they were tested, the subjects sat quietly for 30 min. Resting blood pressure and resting heart rate were measured in duplicate with the subjects in an upright sitting position, and the values of the second measurement were adopted. All participants were instructed not to eat and not to drink fluids other than water for at least 12 h before sampling of blood (*29*). In addition, we checked to be sure that participants did not intake any dietary sources of NOx over the 24 h prior to testing in both groups, since NOx can be affected by diet. The measurements after the exercise training program were performed after subjects had rested for at least 1 day in order to rule out any acute effects from the most recent bout of exercise. Thus we were able to rule out both acute effects from the most recent bout of exercise and oral sources of NOx other than NO. All of the measurements were performed at a constant room temperature (25 °C).

Exercise Test and Exercise Training in Subjects

The subjects performed a symptom-limited ramp-fashion cycling exercise (after 2 min at 20 W, with 15-W increases every 1 min) until they felt exhausted or reached 85% of the age-predicted maximal heart rate, before and after the exercise training program or sedentary program. Their individual VT was calculated using regression analysis of the slopes of CO_2 production, O_2 uptake, and the minute ventilation plot (*32–34*). The work rate of the cycle ergometer for the exercise training was set at 80% of the individual VT level, and was reset using the heart rate corresponding to the initial

Table 1. Effects of Exercise Training or Sedentary Lifestyle in Older Women

Parameter	Sedentary		Training	
	Before	After	Before	After
Height (cm)	153±10	—	152±4	—
Body weight (kg)	53±8	53±8	53±5	53±5
Body mass index (kg/m^2)	23±1	23±2	23±3	23±2
Heart rate (bpm)	77±6	75±6	75±8	71±7
Systolic BP (mmHg)	126±5	130±6	124±13	112±9**,††,§
Diastolic BP (mmHg)	73±3	72±5	73±11	66±6*
Total cholesterol (mg/dl)	217.0±7.3	219.4±15.3	223.0±18.5	217.3±15.6
HDL cholesterol (mg/dl)	53.4±12.4	53.8±14.3	52.6±13.5	61.6±17.5
LDL cholesterol (mg/dl)	122.2±18.3	120.6±17.3	128.2±25.9	128.1±23.8
Triglyceride (mg/dl)	121.4±18.4	123.4±28.5	117.0±39.8	85.8±33.5*,†
Serum insulin (μU/ml)	8.2±1.0	8.7±1.7	8.3±2.5	8.3±2.7
Plasma glucose (mg/dl)	97.4±7.8	101.6±7.0	99.5±13.9	94.6±12.7
HOMA-R	2.0±0.3	2.2±0.5	2.1±0.8	1.9±0.7

BP, blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; HOMA-R, insulin resistance from homeostasis model assessment. Data are means±SD. Significantly different from before exercise training: * $p<0.05$ and ** $p<0.01$. Significantly different from after sedentary life style: † $p<0.05$ and †† $p<0.01$. Significantly different from before sedentary life style: § $p<0.05$.

80% VT level every week. This exercise intensity (80% VT) is probably ～50% of maximal oxygen consumption ($V_{O2\,max}$).

Measurement of NOx Level in the Plasma

The plasma NOx level was determined according to the methods described by our laboratory (*29*) and by Green *et al*. (*35*). Briefly, 80 μl of each sample was incubated for 60 min at 25 °C in a 270 μl incubation mixture containing 140 μl of 125 mmol/l KPi (pH 7.5), 10 μl of 87.5 μmol/l FAD (Sigma, St. Louis, USA), 10 μl of 3.5 mmol/l NADPH, 90 μl of DDW and 20 μl nitrate reductase (1.75 U/ml; Sigma). The reaction was initiated by addition of the nitrate reductase to convert nitrate to nitrite. The reaction was terminated by the addition of 0.8 ml of Griess reagent and 0.45 ml of DDW. After each mixture was centrifuged at 14,000×g for 5 min, the supernatants obtained were determined spectrophotometrically at 542 nm.

Measurement of the cGMP Level in Plasma

The plasma concentration of cGMP was measured by radioimmunoassay after succinylation (*36*).

Statistics

Data are expressed as the means±SD. To evaluate differences in the levels among before and after each program, statistical analysis was carried out by analysis of variance followed by Fisher's protected least significant difference test for multiple comparisons. $p<0.05$ was accepted as significant.

Results

Table 1 shows the characteristic parameters in the older women before and after the 3 months of aerobic exercise training or sedentary lifestyle. There were no significant differences in body weight or body mass index before and after the exercise training (Table 1). SBP and DBP at rest significantly decreased after the exercise training, whereas heart rate at rest was not affected (Table 1). Serum triglyceride concentration significantly decreased after the exercise training (Table 1). There were no significant changes in serum concentrations of total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, or insulin, plasma glucose concentration, or relative insulin resistance from HOMA before and after the exercise training (Table 1). There were no significant differences in any of the characteristic parameters before and after the sedentary lifestyle (Table 1). After the exercise training, individual VT during the exercise test were significantly increased (Fig. 1). These results suggest that the 3-month exercise training in the older women produced favorable physiological effects, as evidenced by the decrease in blood pressure at rest, the decrease in serum triglyceride concentration, and the increase in individual VT during the cycle exercise test. Figure 2 shows the resting plasma NOx concentration in the older women before and after the exercise training or sedentary lifestyle. The plasma concentration of NOx increased significantly after the exercise training (Fig. 2). The plasma cGMP concentration also increased significantly after the exercise training (Fig. 3). There was a significant positive correlation between the %change in plasma NOx concentration and the %change in plasma cGMP concentration

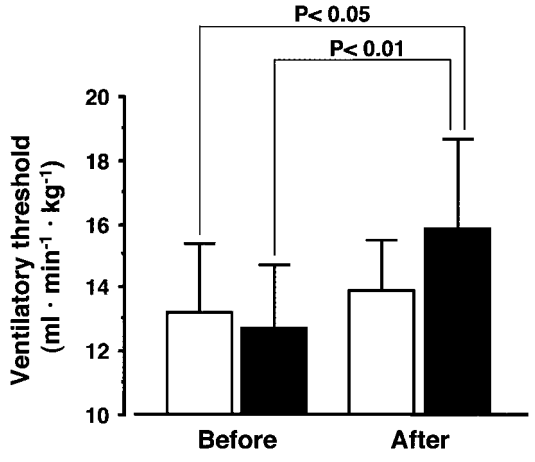

Fig. 1. *Individual ventilatory threshold (VT) during the cycle exercise test before and after a 3-month exercise training (*n=*10; solid bars) or sedentary lifestyle (*n=*5; open bars) program in older women. Data are expressed as the means ±SD.*

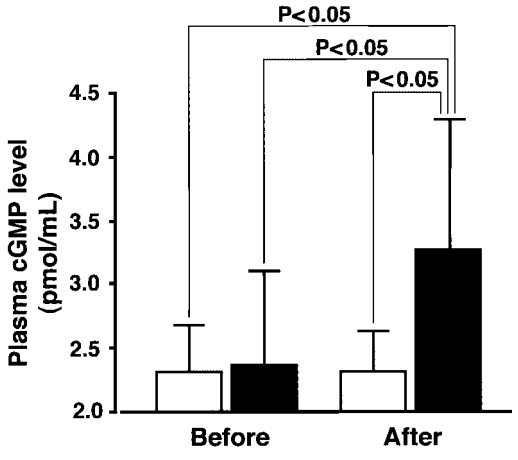

Fig. 3. *The venous plasma concentration of cyclic guanosine monophosphate (cGMP) before and after a 3-month exercise training (*n=*10; solid bars) or sedentary lifestyle (*n=*5; open bars) program in older women. Data are expressed as the means±SD.*

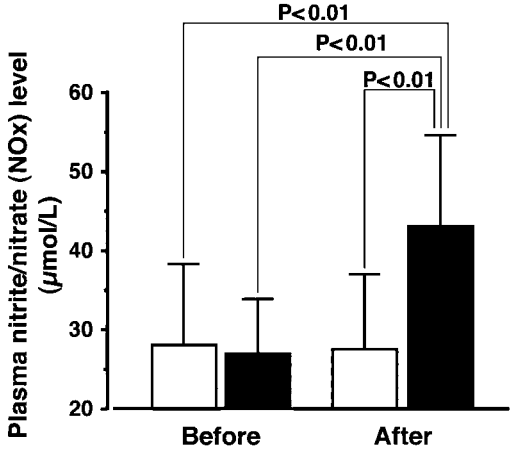

Fig. 2. *The venous plasma concentration of nitrite/nitrate (NOx: the stable end product of NO) before and after a 3-month exercise training (*n=*10; solid bars) or sedentary lifestyle (*n=*5; open bars) program in older women. Data are expressed as the means±SD.*

($r=0.60$, $p<0.05$), and there was a significant negative correlation between the %change in plasma NOx concentration and the %change in SBP ($r=-0.58$, $p<0.05$).

Discussion

In the present study, we measured the plasma concentrations of both NOx (the stable end product of NO) and cGMP (a second messenger of NO) before and after mild exercise training in older women. After the 3 months of exercise due to which the VT significantly increased, the plasma NOx and cGMP concentrations significantly increased. We demonstrated for the first time that endogenous NO production increases in response to a regular, mild aerobic-exercise regimen in previously sedentary older women. This phenomenon may have beneficial effects (*i.e.*, antihypertensive and antiatherosclerotic effects by endogenous NO) on the cardiovascular system.

The older women participated in a regimen of mild exercise training (cycling at 80% VT for 30 min, 5 days/week), which was probably below 50% $V_{O_2\,max}$, in the present study. We previously reported that the plasma NOx concentration was significantly increased by a more intense aerobic exercise training (cycling at 70% $V_{O_2\,max}$ for 1 h, 3–4 days/week) in healthy young humans (*29*). Furthermore, it has been reported that intense aerobic exercise training is associated with improved NO bioavailability in elderly athletes (*30*). In this study, the plasma NOx concentration in the older women increased significantly after the mild exercise training. Thus, it is considered that even mild exercise training could alter the endothelial function in previously sedentary older humans.

NO is produced from L-arginine by NO synthase (NOS) in the vascular endothelium (*11*). In the present study, the plasma NOx and cGMP concentrations were significantly increased after the exercise training. It is possible that the exercise training in the previously sedentary old women ameliorated the deterioration of endothelial function, which may have been induced by aging and/or a sedentary lifestyle, through an up-regulation of the NO system. However, it is also possible that the observed increase in plasma NOx concentration after the exercise training might not be derived from the vascular endothelium, and that the increased NO might be not equivalent to bioactive NO. Although the plasma cGMP concentration was also elevated after the exercise

training, this second messenger is not specific for NO production. Animal studies, however, have reported that exercise training increased the expression of endothelial NOS (eNOS) mRNA in the dog aorta (*37*) and the expression of eNOS protein in the rat aorta (*38*). We also demonstrated in aged rats that the expressions of eNOS mRNA and eNOS protein in the aorta were increased by exercise training (*39*). Furthermore, it has been shown that aerobic exercise training improves NO bioavailability in older adults (*30*, *40*). Thus, the results of the present study are consistent with the idea that repeated bouts of exercise can produce an up-regulation of eNOS in humans, which in turn may provide enhanced synthesis and release of NO, thereby inducing an increase in the plasma NOx level.

The mechanisms by which exercise training enhances NO production are not known. Exercise induces an integrated physiological response, *e.g.*, an increase in circulating neurohumoral factors (*41*), which may exert an influence on NO production (*11*). There is, however, no direct evidence to support the involvement of these substances in the long-term regulation of NO biosynthesis. We recently demonstrated that regular exercise significantly decreased the concentration of plasma endothelin-1 (ET-1), a potent vasoconstrictor peptide produced by vascular endothelial cells, in healthy older humans (*42*). It has been reported that the NO production and ET-1 production pathways engage in cross talk (*43*). Therefore, it is possible that the decrease in ET-1 production by exercise training contributes to the increased production of NO by regular exercise in older women. Furthermore, it is also considered that the increased blood flow velocity induced by exercise may elicit endothelial shear stress, and thereby increase NO production. It has been reported that mechanical deformation of the endothelium by defined shear or cyclic stretching increases eNOS gene expression, proteins, and activity *in vitro* (*44*, *45*). Increased hemodynamic shear stress and/or endothelial stretching induced by repeated bouts of exercise could enhance the long-term biosynthesis of endothelial NO, thereby causing an increase in the plasma NOx concentration after chronic exercise. Alternatively, the following scenario is also possible. Endothelial function is known to be affected by a number of lipid and glycidic parameters. Both LDL cholesterol and triglyceride-rich lipoproteins have been shown to impair NO-dependent vasodilation (*46*), whereas HDL was found to increase eNOS protein expression in cultured human vascular endothelial cells (*47*). In the present study, triglyceride significantly decreased after the aerobic exercise training. Therefore, it is possible that the effect of exercise training on NO may not be direct, but mediated by positive metabolic changes. On the other hand, there is a strong association between insulin resistance and endothelial dysfunction. It has been reported that insulin-resistant humans have impaired endothelium-dependent vasodilator responses (*48*). In the present study, there was no significant difference in insulin resistance from HOMA before and after the exercise training. Therefore, it is possible that the ability of regular aerobic exercise to increase NO production in this population does not depend on changes in insulin resistance. The mechanisms of NO production by exercise training remain to be elucidated.

The present study showed that the plasma concentrations of NOx and cGMP were significantly elevated after a 3-month regimen of exercise training in older women. We previously reported that the plasma NOx concentration was significantly increased by intense aerobic exercise training of 8 weeks in healthy young humans (*29*). Kingwell *et al*. (*49*) showed that 4 weeks of cycle training increased the plasma NOx concentration in humans. Therefore, it is possible that the plasma concentrations of NOx and cGMP are elevated early in the exercise training. However, it is unclear whether the plasma concentrations of NOx and cGMP were increased early in the exercise training in the present study, because plasma NOx and cGMP concentrations may be affected the intensity and/or time of exercise, and/or the age of subjects. The duration of exercise training needed to induce significant alterations of plasma NOx and cGMP concentrations by mild exercise in the elderly remains to be elucidated.

It is well known that regular exercise produces beneficial effects on the cardiovascular system (*50*–*57*). Chronic exercise reduces blood pressure in patients with moderate hypertension (*51*–*53*). Furthermore, it has been reported that even mild exercise training (training at 50% $V_{O_2\,max}$), which is similar to the present training intensity, decreased blood pressure in subjects with mild-to-moderate hypertension (*54*). The aging-induced reduction in arterial compliance causes an increase in SBP, whereas exercise training prevents this reduction in arterial compliance (*55*, *56*). It has also been reported that exercise training has a favorable effect on the development of atherosclerosis (*57*). However, the precise mechanisms by which exercise training produces these beneficial effects on the cardiovascular system have not been fully determined. The present study demonstrated that the plasma NOx and cGMP concentrations significantly increased after exercise training in older humans. We also observed a reduction in blood pressure after exercise training in the older women, along with increases in plasma NOx and cGMP. NO has potent vasodilative and antiatherosclerotic effects (*11*, *12*). Therefore, the increase in production of endogenous NO in endothelial cells by exercise training may have been partly involved in the exercise training-induced beneficial effects on the cardiovascular system in older humans in the present study.

In summary, we demonstrated that the plasma concentrations of NOx and cGMP significantly increased after mild aerobic-endurance exercise training in previously sedentary older women. Because NO has been implicated in the moderation of vascular tonus and the prevention of progression of atherosclerosis, we propose that the increase in the production of NO by exercise training may be partly involved in the beneficial effects of chronic exercise on the cardiovascular system in older humans. The beneficial effects on the car-

diovascular system induced by comparatively mild exercise training may have great significance for health promotion in previously sedentary older humans.

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