Original Article

Blood Pressure-Lowering Effects of Lifestyle Modification: Possible Involvement of Nitric Oxide Bioavailability

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Lifestyle modification is recommended as a non-pharmacological approach to treatment of hypertension. Many investigators have reported that exercise has antihypertensive effects, and various mechanisms have been proposed to explain this phenomenon. For example, nitric oxide (NO), which may be increased by exercise, has been reported to play a crucial role in preserving vessel homeostasis both by regulating vascular tone and by exerting anti-atherosclerotic effects. NO is known to be exquisitely sensitive to inactivation by superoxide radicals. However, the relationship between the blood pressure-lowering effect of lifestyle modification and NO bioavailability remains unknown. We investigated the effects of a 12-week lifestyle modification program consisting of mild exercise and diet on changes in blood pressure, plasma nitrate/nitrite (NOx), plasma nitrotyrosine, which is the footprint of NO interaction with reactive oxygen species, and plasma extracellular-superoxide dismutase (EC-SOD). The 12-week lifestyle modification program lowered blood pressure and increased plasma NOx. When the subjects were divided into two groups according to the change of plasma nitrotyrosine as an indicator of NO bioavailability, the subjects whose plasma nitrotyrosine decreased exhibited a significant relationship between the blood pressure-lowering effect of the lifestyle modification and the increase in EC-SOD, whereas those without a decrease in plasma nitrotyrosine exhibited a significant relationship between the blood pressure-lowering effect and the increase in maximum oxygen consumption. These results indicate that the level of NO bioavailability influences the mechanism of the blood pressure-lowering effect of aerobic exercise and diet. (*Hypertens Res* 2005; 28: 779–786)

***Key Words*: nitric oxide bioavailability, oxidative stress, lifestyle modification, blood pressure, extracellular superoxide dismutase**

Introduction

A number of studies have indicated that oxidative stress is closely associated with the pathogenesis of lifestyle-related diseases, including hypertension (*1*, *2*), hyperlipidemia (*3*), and obesity (*4*, *5*). Superoxide dismutases (SODs) help to defend cells against superoxide radicals (*4*). There are three isozymes of SODs: a cytosolic copper/zinc-containing form (Cu/Zn-SOD), a mitochondrial manganese form (Mn-SOD),

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and an extracellular form (EC-SOD) that is also a copper/zinc-containing enzyme. Since EC-SOD is highly expressed in blood vessels and is the predominant form of SOD in vascular tissues (*6*), EC-SOD has been reported to play a major role in the prevention of oxidative stress on the surface of vascular cells (*7*).

Lifestyle modification is recommended as a non-pharmacological approach to the treatment of hypertension (*8*, *9*). Regular aerobic exercise has a number of beneficial effects on the reduction of cardiovascular risk factors (*10*, *11*). One of the possible effects of mild exercise is to induce mild oxidative stress that stimulates the induction of certain antioxidant enzymes (*5*, *12*). There are also some evidences that physical exercise, which exerts a beneficial effect on the reduction of cardiovascular morbidity and mortality (*13*, *14*), induces the expression of antioxidant enzymes such as Cu/Zn-SOD and Mn-SOD (*15*). Fukai *et al*. also reported that treadmill exercises increased EC-SOD expression in mice (*16*). Taddei *et al*. have shown that regular physical activity as well as supplementation of non-enzymatic antioxidants such as vitamin C restored nitric oxide (NO) bioavailability by reducing oxidative stress (*17*). There has also been a report that regular exercise improved endothelial function in patients with mild hypertension (*18*).

On the other hand, Taddei *et al*. reported that in healthy subjects, the vasodilation induced by acetylcholine was similar between young trained subjects and young sedentary subjects, whereas the response to acetylcholine was significantly greater in elderly trained subjects than in elderly sedentary subjects (*19*). In their review, Green *et al*. suggested that exercise training of healthy subjects might not improve NO bioavailability, and that improvement of NO bioavailability might be possible in those with antecedent endothelial dysfunction (*20*). So, it is not necessarily the case that lifestyle modification consisting of mild exercise and diet improves NO bioavailability, especially in middle-aged subjects.

NO plays a crucial role in preserving vessel homeostasis, both by regulating vascular tone and by exerting anti-atherosclerotic effects. NO is known to be exquisitely sensitive to inactivation by superoxide radicals (*21*). The reaction of superoxide and NO forms peroxynitrite, a strong oxidant, which in turn reacts with free tyrosine or tyrosine residues in protein molecules to produce nitrotyrosine (*22*). Alternatively, reactive oxygen species (ROS) produce tyrosine radicals, which react with NO to produce nitrotyrosine (*23*). Thus, nitrotyrosine is generally considered to be footprint of NO-ROS interaction (*24*), which is an indicator of NO bioavailability. Since the regulation of extracellular superoxide concentrations is important in preserving the biological activity of NO, EC-SOD is considered to be a key regulator of NO bioavailability (*25*). In patients with essential hypertension, the production of ROS is one of the primary mechanisms leading to impaired endothelium-dependent vasodilatation *via* the reduction of NO bioavailability (*17*).

The present study was designed to assess the relationship between the blood pressure-lowering effects of exercise and healthy diet and the increase of NO bioavailability due to the quenching of superoxide radicals by EC-SOD. For this purpose, we investigated changes in blood pressure and plasma nitrotyrosine as an indicator of NO bioavailability in 132 subjects during a lifestyle modification program consisting of mild aerobic exercise and diet counseling.

Methods

Subjects

Two hundred seventy subjects voluntarily participated in a 12-week lifestyle modification program focused on the prevention of lifestyle-related diseases conducted by the Kitakyushu Municipal Health Promotion Committee. Two hundred forty-three of these subjects provided written informed consent to participate in the present study, and 111 of these were excluded because post-intervention data was not available ($n=70$) or because they were being treated with an antihypertensive drug and/or other medication, such as a statin or an anti-inflammatory drug ($n=41$). The final study group thus consisted of 132 subjects (39 men, 93 women; mean age, 52.2±9.8 years); their profiles are summarized in Table 1. The averages of systolic and diastolic blood pressure (SBP and DBP) were 129.1±17.1 and 78.8±11.2 mmHg, respectively. Thirty-six subjects were mild to moderate hypertensives, and other 96 subjects were normotensives. The program consisted of mild aerobic exercise and diet counseling. Blood pressure was measured in a sitting position twice between 2:00 PM and 6:00 PM with an automatic sphygmomanometer (BP-203RV; Nihon Colin, Tokyo, Japan), and the blood pressure of individual participants was taken as the mean of the two-recorded readings. This study was approved by the ethics committee of the University of Occupational and Environmental Health. The authors obtained the written informed consent of each participant before the start of the program.

Protocol for Lifestyle Modification Program

Exercise Training

To estimate maximum oxygen consumption ($\dot{V}_{O_2\,max}$) and to determine a suitable exercise intensity for mild exercise, a four-stage graded submaximal test was performed using a cycle ergometer (ML-1800; Fukuda Denshi, Tokyo, Japan).

The mild exercise consisted of a 10-min stretching session followed by 30 min on a cycle ergometer or walking indoors, and then 30 min of low-impact aerobics. Subjects were instructed to check their own pulse rate on their radial artery and to exercise at a prescribed pulse rate corresponding to an intensity of 50% $\dot{V}_{O_2\,max}$ or the anaerobic threshold point. Registered trainers and doctors supervised the exercises and the subjects were advised to maintain their target heart rate. The subjects participated in the above exercise sessions twice per

Table 1. Summary of Effect of Lifestyle Modification Program

Variables	Before	After	*p* value
BMI (kg/m^2)	23.7±2.9	23.3±2.7	<0.0001
SBP (mmHg)	129.1±17.1	125.3±16.9	0.0010
DBP (mmHg)	78.8±11.2	76.5±10.3	0.0010
$\dot{V}_{O_2\,max}$ (ml/kg/min)	32.7±6.3	34.7±6.3	<0.0001
FPG (mg/dl)	98.3±8.8	98.0±9.0	0.7269
HDL cholesterol (mg/dl)	64.4±14.5	66.3±14.7	0.0040
Total cholesterol (mg/dl)	217.0±33.4	212.2±35.7	0.0076
Triglyceride (mg/dl)	115.0±85.7	107.3±78.8	0.0812
LDL cholesterol (mg/dl)	129.5±33.8	124.5±32.5	0.0026
Plasma NOx (μmol/l)	65.4±25.7	75.2±34.2	0.0484
Plasma EC-SOD (AU)	134.3±59.8	137.0±63.9	0.2812
Plasma nitrotyrosine (AU)	116.4±61.0	118.2±53.9	0.4556
Plasma TBARS (μmol/l)	1.38±0.41	1.33±0.42	0.0686
Dietary data			
Total calorie intake (kcal/day)	1,945.4±529.4	1,814.0±435.9	0.0007
Salt intake (g/day)	12.6±3.4	11.7±2.8	0.0044
VitA intake (IU/day)	2,767.7±1,175.3	3,134.0±1,308.0	0.0012
VitC intake (mg/day)	181.8±76.9	202.0±83.5	0.0002
VitE intake (mg/day)	10.1±2.7	10.3±2.7	0.7802
Dietary fiber intake (g/day)	19.6±7.0	20.9±7.3	0.0089

Wilcoxon's test. Values in the table are mean±SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL cholesterol, high density lipoprotein cholesterol; LDL cholesterol, low density lipoprotein cholesterol; NOx, nitrate/nitrite; EC-SOD, extracellular superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; AU, arbitrary unit; Vit, vitamin.

week. The information about daily physical activity during the intervention was obtained from self-administered questionnaires before and after the intervention.

Diet

Subjects completed a self-administered questionnaire on dietary habits (WELL-200; Fukuda Denshi) before and after the intervention (*11*). Based on the information before the intervention, personal diet counseling was provided by registered dietitians at the baseline and 6 weeks thereafter. Such diet counseling generally included instructions not to eat snacks, to limit alcohol and/or high-calorie beverage consumption, to reduce fatty foods, and to restrict salt intake. Four to eight objectives were then established for each subject. The subjects were encouraged to meet their objectives, and kept a daily record of their progress.

Measurement of Plasma Biochemical Parameters

Blood samples were collected after an at least 12-h nocturnal fasting. Plasma thiobarbituric acid-reactive substances (TBARS), an indicator of lipid peroxide, was assayed by the fluorescence method, as described previously (*26*, *27*). In the present study, we adopted plasma nitrite/nitrate (NOx) levels as an indicator of NO production, since this study was focused on the NO bioavailability in relation to nitrotyrosine levels in plasma. Plasma levels of NOx were measured using an auto-analyzer system equipped with a copperized cadmium reduction column to reduce nitrate to nitrite (Tokyo Kasei Kogyo Co., Tokyo, Japan), as described previously (*28*, *29*). The sum of nitrite and nitrate was taken to be NOx. Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, and fasting plasma glucose (FPG) were measured and low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula (LDL cholesterol = total cholesterol − HDL cholesterol − 1/5 × triglyceride).

Measurement of Plasma Nitrotyrosine and EC-SOD

Plasma levels of nitrotyrosine and EC-SOD were measured by the Western blotting method, as described previously (*30*). An equal amount of each sample was subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE; 12% gels). The separated proteins were then transferred to nitrocellulose membranes and probed with mouse monoclonal antibody to nitrotyrosine (Zymed Laboratories Inc., San Francisco, USA) or rabbit polyclonal antibody to EC-SOD (produced by Sigma-Aldrich Japan K.K. [Tokyo, Japan] using the oligopeptides CTGEDSAEPNSD, corresponding to residues 20–30 of the human EC-SOD protein (*31*)). A horse radish peroxidase (HRP)-conjugated second-

Table 2. Baseline Data and Effect of Lifestyle Modification of Subjects with or without a Decrease in Plasma Nitrotyrosine after the Intervention

Valuables	pNT-decreased group (n=65)			pNT-non-decreased group (n=67)		
	Before	After	p value	Before	After	p value
Gender (men/women)	24/41			15/52		
Age (years)	51.5±9.8			52.8±9.9		
Frequency (counts/12-week)	20.9±5.6			21.3±4.6		
BMI (kg/m^2)	24.1±3.0	23.8±2.7	<0.0001	23.2±2.8	22.8±2.6	<0.0001
SBP (mmHg)	126.8±15.9	122.6±14.9	0.0139	131.3±18.0	127.9±18.3	0.0285
DBP (mmHg)	78.6±11.3	75.6±9.1	0.0026	79.0±11.1	77.4±11.4	0.0974
$\dot{V}_{O_2\,max}$ (ml/kg/min)	32.8±5.9	34.7±6.4	<0.0001	32.7±6.7	34.6±6.3	<0.0001
FPG (mg/dl)	99.3±9.5	98.3±8.8	0.3954	97.3±8.1	97.8±9.3	0.7122
HDL cholesterol (mg/dl)	62.0±13.8	64.7±14.4	0.0003	66.8±14.9	67.8±15.0	0.3741
Total cholesterol (mg/dl)	213.3±34.3	211.5±37.2	0.5584	220.6±32.5	212.9±34.5	0.0018
Triglyceride (mg/dl)	110.3±55.9	100.0±50.8	0.0356	119.6±107.2	114.3±98.6	0.7062
LDL cholesterol (mg/dl)	129.2±30.6	126.8±33.7	0.4140	129.8±36.8	122.2±31.4	0.0007
Plasma NOx (μmol/l)	65.4±26.8	77.3±35.6	0.0937	65.4±24.9	73.1±32.9	0.2466
Plasma EC-SOD (AU)	128.2±50.9	132.4±59.4	0.4484	140.1±67.1	141.6±68.1	0.4276
Plasma nitrotyrosine (AU)	129.6±72.5	102.3±55.8	<0.0001	103.6±44.4	133.7±47.5	<0.0001
Plasma TBARS (μmol/l)	1.34±0.39	1.31±0.41	0.3946	1.42±0.43	1.35±0.44	0.0881
Dietary data						
Total calorie intake (kcal/day)	1,998.7±588.5	1,845.6±417.6	0.0334	1,893.7±463.7	1,783.3±453.9	0.0081
Salt intake (g/day)	12.7±3.4	11.7±2.8	0.0216	12.5±3.5	11.6±2.9	0.0840
VitA intake (IU/day)	2,587.0±1,115.8	3,105.1±1,302.4	0.0006	2,942.9±1,212.8	3,161.9±1,322.6	0.2161
VitC intake (mg/day)	166.5±72.6	195.4±86.7	0.0005	196.7±78.5	208.3±80.5	0.0836
VitE intake (mg/day)	10.0±2.6	10.3±2.8	0.3206	10.3±2.8	10.2±2.6	0.5524
Dietary fiber intake (g/day)	18.2±6.6	20.2±7.4	0.0065	21.0±7.2	21.6±7.3	0.3237

pNT-decreased group, whose plasma nitrotyrosine decreased, and pNT-non-decreased group, whose plasma nitrotyrosine increased or unchanged. Wilcoxon's test. Values in the table are mean±SD. p value for pNT-decreased group *vs.* pNT-non-decreased group. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL cholesterol, high density lipoprotein cholesterol; LDL cholesterol, low density lipoprotein cholesterol; NOx, nitrate/nitrite; EC-SOD, extracellular superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; AU, arbitrary unit; Vit, vitamin.

ary antibody was used in conjunction with an enhanced chemiluminescence detection kit (ECL) from Amersham Pharmacia Biotech (Little Chalfont, UK) to visualize the immunoreactive bands on autoradiography film. The intensity of the immunoreactive band was quantified by densitometric analysis using the computer program Doc-It System (UVP Inc., Upland, USA).

Statistical Analysis

All statistical calculations were performed with personal computer statistical software (STATVIEW 5.0; SAS Institute Inc., Cary, USA). Differences in mean values were assessed by Mann-Whitney's *U*-test or Wilcoxon's test for comparison of two variables. We compared the categorical variables between groups by using the χ^2 test. To assess the independent determinants of the blood pressure lowering-effect of the intervention, we employed multiple stepwise regression analysis, adopting the percent basal (%basal) value of blood pressure as a dependent variable and various other variables as independent variables. The %basal value was calculated by the following formula: (value after the intervention) / (value before the intervention) × 100. All results are expressed as the mean±SD. Values of $p<0.05$ were considered to indicate statistical significance.

Results

The Effects of the Lifestyle Modification Program on Physiological, Biochemical, and Dietary Parameters

The effects of the lifestyle modification program are summarized in Table 1. Body mass index (BMI), blood pressure, total cholesterol, and LDL cholesterol were significantly decreased, and $\dot{V}_{O_2\,max}$, HDL cholesterol, and plasma NOx were significantly increased by the intervention. As for dietary parameters, total calorie and salt intake were significantly decreased, and intake of antioxidant vitamins such as vitamin A and C, and dietary fiber were significantly

Table 3. Results of Multiple Stepwise Regression Analysis for Dependent Variable of Systolic Blood Pressure (%Basal)

Variables	pNT-decreased group		pNT-non-decreased group	
	β	F	β	F
Gender	0.092	0.530	0.031	0.059
Age	0.109	0.744	0.044	0.124
Frequency	0.089	0.499	−0.114	0.833
BMI (%basal)	0.117	0.858	−0.080	0.403
$\dot{V}_{O_2\,max}$ (%basal)	−0.235	3.608	−0.270	5.032*
FPG (%basal)	−0.048	0.141	0.203	2.698
LDL cholesterol (%basal)	0.018	0.020	0.081	0.412
Plasma NOx (%basal)	0.083	0.433	−0.126	1.012
Plasma EC-SOD (%basal)	−0.256	4.426*	−0.192	2.419
Plasma TBARS (%basal)	−0.032	0.064	−0.039	0.096
Dietary data				
Total calorie intake (%basal)	0.155	1.535	0.048	0.147
Salt intake (%basal)	−0.069	0.295	0.132	1.121
VitA intake (%basal)	−0.063	0.247	−0.028	0.051
VitC intake (%basal)	−0.194	2.436	0.142	1.288
VitE intake (%basal)	−0.164	1.708	0.082	0.423
Dietary fiber intake (%basal)	−0.166	1.764	0.181	2.122

*$F>4$ for significance $p<0.05$. pNT-decreased group, whose plasma nitrotyrosine decreased, and pNT-non-decreased group, whose plasma nitrotyrosine increased or unchanged. The %basal value = (value after the intervention)/(value before the intervention)×100. BMI, body mass index; FPG, fasting plasma glucose; HDL cholesterol, high density lipoprotein cholesterol; LDL cholesterol, low density lipoprotein cholesterol; NOx, nitrate/nitrite; EC-SOD, extracellular superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; Vit, vitamin.

increased by the intervention.

Relationships between the Changes in NO Bioavailability and Blood Pressure-Lowering Effect

In order to clarify the relationship between cardiovascular risk factors, including blood pressure, and the change in NO bioavailability, we divided the subjects into two groups according to the difference of the change in plasma nitrotyrosine by the intervention: a pNT-decreased group whose plasma nitrotyrosine decreased, and a pNT-non-decreased group whose plasma nitrotyrosine increased or was unchanged.

At the baseline of the intervention, there were no differences between the two groups in any of the variables except for HDL cholesterol (62.0±13.8 mg/dl for the pNT-decreased group *vs.* 66.8±14.9 mg/dl for the pNT-non-decreased group, $p=0.0219$) and dietary fiber intake (18.2±6.6 g/day for the pNT-decreased group *vs.* 21.0±7.2 g/day for the pNT-non-decreased group, $p=0.0206$). Moreover, there were no significant differences in the plasma levels of nitrotyrosine or the frequency of participation in exercise training between the two groups (Table 2). There were also no significant differences in smoking status or daily physical activities between the two groups.

As shown in Table 2, BMI and $\dot{V}_{O_2\,max}$ were improved to similar extents in both groups. The smoking status was not changed by participation in this program, and daily physical activities were similarly increased in both groups. The intervention significantly reduced DBP only in the pNT-decreased group, although SBP was significantly decreased in both groups. The intervention also significantly decreased triglyceride and increased HDL cholesterol in the pNT-decreased group, and decreased total cholesterol and LDL cholesterol in the pNT-non-decreased group. As for dietary parameters, in the pNT-decreased group the total calorie intake and salt intake were decreased, and the intake of antioxidant vitamins such as vitamin C and A and dietary fiber were increased by the intervention, whereas in the pNT-non-decreased group only total calorie intake was decreased.

To verify whether the independent determinants of the blood pressure-lowering effect were different between the two groups, we carried out multiple stepwise regression analysis separately in the pNT-decreased and non-decreased groups. In the multiple stepwise regression analysis, we adopted the %basal value of SBP compared to a basal value as a dependent variable, and gender, age, frequency, and %basal of BMI, $\dot{V}_{O_2\,max}$, FPG, LDL cholesterol, plasma NOx, plasma EC-SOD, TBARS, total calorie intake, salt intake, vitamin A, C, E intake, and dietary fiber intake compared to a basal value as independent variables, as shown in Table 3. There was an inverse association between the %basal value of plasma EC-SOD and the %basal value of SBP in the pNT-decreased group, whereas an inverse association was observed between

the %basal value of $\dot{V}_{O2\,max}$ and the %basal value of SBP in the pNT-non-decreased group.

Discussion

Arakawa and his colleagues extensively investigated the beneficial effects of exercise therapy on hypertension and proposed various physiological and biochemical mechanisms for the antihypertensive effects of exercise, including improvement of sympathetic regulation and volume-depleting actions (*32*). Recently, chronic exercise training using animal models has been shown to improve acetylcholine-induced vasodilatation probably due to the increase in NO production (*33*). Sessa *et al*. showed that exercise increased the expression of endothelial nitric oxide synthase (eNOS) in dog coronary arteries (*34*). In a study on humans, Maeda *et al*. reported that plasma levels of NOx were increased by mild exercise in elderly women (*35*). However, increased NO production does not always result in improved physiological function. NO is highly reactive with superoxide, forming peroxynitrite, a potent oxidant. Once NO is released into the extracellular space where superoxide is being formed, it is easily inactivated by superoxide. Therefore, the amount of superoxide relative to NO plays a key role in the maintenance of NO bioavailability. Nitrotyrosine is a footprint of NO-ROS interaction, and is considered an indicator for NO inactivation (*24*). Growing evidence has been accumulating to indicate that chronic exercise enhances antioxidant defense systems in various mammalian tissues (*36*, *37*). In the present study, the subjects who exhibited a decrease in plasma nitrotyrosine showed a relationship between the increase in EC-SOD and the blood pressure-lowering effect of lifestyle modification after adjustment for other factors. These results suggested that the blood pressure-lowering effect could be attributed in part to the increase in NO bioavailability due to the suppression of peroxynitrite production mediated by the quenching of superoxide by EC-SOD.

Since EC-SOD is highly expressed and is one of the most prevalent forms of SOD in the vascular walls, it likely plays a critical role in the defense against oxidative stress and the modulation of NO bioavailability in vascular tissues. A study using mice lacking EC-SOD indicated that EC-SOD played an important role in the prevention of NO consumption by superoxide (*38*). Hornig *et al*. reported that pharmacological interference with the renin-angiotensin system in human subjects improved endothelial function by increasing NO bioavailability, which may be mediated in part by increased EC-SOD activity (*39*). On the other hand, it is possible that NO *per se* increases the expression of EC-SOD, since Fukai *et al*. demonstrated in an animal model that exercise training increased EC-SOD expression mediated by endothelium-derived NO (*16*). Further studies will be needed to establish the relationship between NO and EC-SOD in humans. On the other hand, Garcia *et al*. reported that intra-arterial infusion of SOD to hypertensive subjects did not improve impaired endothelium-dependent vasodilation (*40*). They used Cu/Zn-SOD that had a low binding affinity to the vascular endothelial surface, which may not exert an effective suppression on oxidative stress at the local milieu of vascular vessels.

Taddei *et al*. have reported on the effects of non-enzymatic antioxidants on NO bioavailability (*17*, *19*, *41*). Since our intervention included diet counseling as well as exercise, changes in non-enzymatic antioxidant intake should be considered. Increase in EC-SOD independently contributed to the blood pressure-lowering effect in the subjects whose plasma nitrotyrosine decreased, although there were significant increases in the intake of vitamin A and C by the intervention according to the analysis of the self-administered questionnaire.

DBP was significantly reduced in all subjects except those whose plasma nitrotyrosine levels were increased or unchanged. Recently, Adachi *et al*. reported that irreversible oxidation of key protein thiols represented a direct cause of decreased NO bioavailability in chronic diseases (*42*). One explanation for our results might be that the blood pressure-lowering effect mediated by NO was attenuated due to a decrease in NO bioavailability in the subjects whose plasma nitrotyrosine was increased or unchanged by the intervention. SBP was decreased by the intervention, and the increase in $\dot{V}_{O2\,max}$ independently contributed to the blood pressure-lowering effect. This result is consistent with a report by Blair *et al*. (*43*). Although the precise blood pressure-lowering mechanisms remain to be elucidated, they might involve the reduction of peripheral vascular resistance by sympathetic regulation (32) and/or other factors such as prostaglandin E (*44*) through an NO-independent pathway. Our results showed that LDL cholesterol was decreased only in the subjects whose plasma nitrotyrosine was increased or unchanged by the intervention. It has been reported that excessive blood pressure responses to stress tests are improved after cholesterol-lowering therapy in hypercholesterolemic patients (*45*). Therefore, an improvement of LDL cholesterol may have been involved in the blood pressure-lowering effect in our subjects who showed no increase in NO bioavailability by the intervention.

In conclusion, the present study showed that a 12-week lifestyle modification consisting of aerobic exercise and diet lowered blood pressure in 132 subjects consisting of 36 hypertensives and 96 normotensives, but differences in NO bioavailability influenced the mechanism of the blood pressure reduction.

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