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Forearm endothelial function and bone mineral loss in postmenopausal women

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Abstract

It is widely believed that the vasculature plays an important role in bone remodeling. We investigated the relationship between forearm endothelial function and bone mass in the lumbar spine in early postmenopausal women without a history of smoking or diabetes mellitus.

We studied the forearm resistance artery endothelial function in 110 Japanese women—52 postmenopausal women with normal spinal bone mineral density (BMD), 36 postmenopausal women with osteopenia, and 22 osteoporotic postmenopausal women. Forearm blood flow (FBF) during reactive hyperemia and after sublingual nitroglycerin (NTG) administration was measured by strain-gauge plethysmography. BMD of the lumbar spine (L2–L4) was measured by dual-energy X-ray absorptiometry. After adjustment for age, body mass index, years since the start of menopause, and basal FBF, women with osteoporosis had a lower maximal FBF response to reactive hyperemia (28.4 ± 3.8 mL/min per 100 mL tissue) than those with normal BMD (39.8 ± 2.8 mL/min per 100 mL tissue) or osteopenia (35.6 ± 2.5 mL/min per 100 mL tissue) ($P = 0.029$). A significant increase in serum angiotensin-converting enzyme (ACE) activity ($P = 0.042$) and a significant decrease in the serum concentrations of nitrite/nitrate ($P = 0.041$) were noted in osteoporotic women compared to women with normal BMD or osteopenia.

The present findings suggest that postmenopausal women with low BMD, especially those with osteoporosis, have impaired endothelial function in the forearm resistance arteries.

Keywords: Bone mineral density; Forearm endothelial function; Angiotensin-converting enzyme; Postmenopausal women; Nitric oxide

1. Introduction

Recent research suggests that bone loss and cardiovascular disease are functionally interwoven [1,2]. Osteoporosis is associated with both atherosclerosis and vascular calcification. Osteoporotic postmenopausal women are at significantly greater risk for cardiovascular disease than are age-matched controls [3]. Patients with low bone density and osteoporosis also have higher lipids level, more severe coronary atherosclerosis, and have a greater risk of stroke death [4–6]. The presence of osteoporotic fractures and low bone mass are major risk factors for future fractures. Bone mineral density (BMD) measurements are often performed to advise women whether to consider preventive therapies for osteoporosis [7]. If the association between cardiovascular disease and low bone mass is real, then women with low bone mass might be treated by a therapy that also prevented cardiovascular disease.

Endothelial dysfunction is thought to be one of the initial stages in the development of atherosclerosis. The endothelium plays a major role in determining vascular tone through the production and release of vasodilators such as nitric oxide (NO), which helps to prevent atherosclerosis by

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maintaining vasodilation and inhibiting platelet aggregation, leukocyte adhesion, and the proliferation of smooth muscle cells [8,9]. However, it is not known whether BMD is associated with vascular endothelial function in postmenopausal women. Furthermore, previous epidemiologic studies [3,10] have lacked rigorous control for smoking history and other cardiovascular risk factors.

To determine the possible association between osteoporosis and cardiovascular disease, we therefore investigated the relationship between BMD in the lumbar spine and endothelial function in early postmenopausal women without a history of smoking or diabetes mellitus.

2. Methods

Of 356 postmenopausal Japanese women who presented to our clinic between 1998 and 2002, 110 subjects (mean age: 53.8 ± 1.4 years) were enrolled randomly. Women were eligible, if menopause had occurred at least 1 year prior to their visit. Menopausal status was confirmed by a serum follicle-stimulating hormone (FSH) concentration exceeding 30 IU/L and a serum estradiol concentration below 20 pg/mL. Each patient was of normal weight, with a body mass index not exceeding 25 kg/m^2. Excluded from the study were patients with a history of tobacco, alcohol, and caffeine use; fractures, diabetes, hypertension, myocardial infarction, liver disorders, and family history of osteoporotic fracture or premature myocardial infarction. None of the patients had undergone hormonal replacement therapy or had taken any steroid hormones or medications known to affect lipid metabolism, blood pressure or bone metabolism. Written, informed consent for participation was obtained from each subject prior to enrollment. The Ethics Committee of the Department of Obstetrics and Gynecology at Hiroshima University approved the study protocol.

Individuals were assigned to one of three groups according to BMD in the lumbar spine—normal BMD (52 women), osteopenia (a value for BMD that was 1–2.5 S.D. below the mean value for young adults, 36 women), and osteoporosis (a value for BMD that was more than 2.5 S.D. below the mean value for young adults, 22 women). An expert panel of World Health Organization defined this classification system [11].

2.1. Measurement of bone mineral density

The BMD in the lumbar spine (L2–L4) was measured by dual-energy X-ray absorptiometry (DXA; model DPX-L; Lunar, Madison, WI). The BMD is reported as grams per square centimeter. All the measurements were performed by two operators, and the same technician analyzed all of the scans. The inter-operator coefficients of variation were 1.7 and 0.8%, which was tested by measuring the BMD twice in 19 of the participants (mean age: 56 ± 5 years), respectively.

2.2. Measurement of forearm blood flow

The vasodilator responses to reactive hyperemia and sublingual nitroglycerine (NTG) in each subject were measured. This evaluation began at 8:30 A.M. Each subject had fasted for at least 14 h, and then rested supine in a quiet, air-conditioned room (constant temperature, 22–25 °C). After 30 min of rest, the basal FBF was measured as described below. Next, the effects of reactive hyperemia and of sublingual NTG administration on FBF were evaluated by inflating a cuff over the left upper arm to 280 mmHg for 5 min. After the cuff occlusion was released, the FBF was measured for 3 min. Next, a NTG tablet (0.3 mg) (Nihonkayaku Co., Tokyo, Japan) was administered sublingually, and the FBF was again measured for 3 min. These studies were carried out in a randomized fashion. Each study proceeded after FBF had returned to baseline. In a preliminary study, we confirmed the reproducibility of the FBF response to reactive hyperemia and sublingual NTG on two separate occasions in 28 healthy male subjects (mean age: 27 ± 5 years). The coefficients of variation were 4.3 and 2.8%, respectively. The FBF was measured with a mercury-filled Silastic strain-gauge plethysmograph (EC-5R, D.E. Hokanson Inc., Issaquah, Washington), as previously described [12,13]. Briefly, the strain-gauge was attached to the left upper arm, supported above the right atrium, and connected to the plethysmography device. A wrist cuff was inflated to 50 mmHg above the systolic blood pressure to exclude hand circulation from the measurements beginning 1 min before each measurement and was continued throughout the determination of FBF. The upper arm cuff was inflated to 40 mmHg for 7 s in each 15 sec 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., Nagoya, Japan). The FBF was expressed as mL/min per 100 mL of forearm tissue volume. The FBF was calculated by two independent observers who had no knowledge of the subject's profile based on linear portions of the plethysmographic recordings. The intraobserver coefficient of variation was 3.0 ± 1.6%. Four plethysmographic measurements were averaged to obtain the FBF at baseline, during reactive hyperemia, and after the administration of sublingual NTG.

2.3. Analytical methods

Samples of venous blood were placed in polystyrene tubes containing sodium EDTA (1 mg/mL) and immediately chilled in an ice bath. Plasma was separated by centrifugation at 3100 rpm at 4 °C for 10 min. Serum was separated at 1000 rpm at room temperature for 10 min. Samples were stored at −80 °C until assayed. Routine chemical methods were used to determine the serum concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides (TG), creatinine, glucose, and electrolytes. The serum concentration of low-density lipopro-

Table 1
Clinical variables in the normal BMD, osteopenia and osteoporosis groups

Variable	Normal BMD	Osteopenia	Osteoporosis	*P*-value
Number	52	36	22	
Age (y)	52.6 ± 4.0	54.7 ± 5.0	59.6 ± 4.3	<0.001
Height (cm)	155.7 ± 4.6	153.9 ± 3.9	149.5 ± 6.9	<0.001
Weight (kg)	56.2 ± 8.3	51.4 ± 4.8	47.9 ± 5.6	<0.001
Body mass index (kg/m^2)	22.9 ± 1.4	22.1 ± 1.6	21.7 ± 1.7	0.136
Years since menopause (y)	3.4 ± 4.1	5.3 ± 4.6	11.9 ± 6.2	<0.001
Basal FBF (mL/min per 100 mL tissue)	6.3 ± 1.9	6.1 ± 2.3	5.8 ± 1.7	0.647

BMD, bone mineral density; FBF, forearm blood flow. All results are presented as mean ± S.D.

tein (LDL) cholesterol was determined by Freidewald's method [14]. Serum concentrations of estradiol were measured by a radioimmunoassay. Nitrite/nitrate concentrations were measured with an autoanalyzer (flow injection analyzer, TCI-NOX1000, Tokyo Kasei Kogyo, Tokyo, Japan), which uses a protocol based on the Griess reaction [15]. Serum angiotensin-concerting enzyme (ACE) activity (in international units per liter at 37 °C) was measured with ACE Color (Fuji Rebio Co. Ltd., Tokyo, Japan) [16].

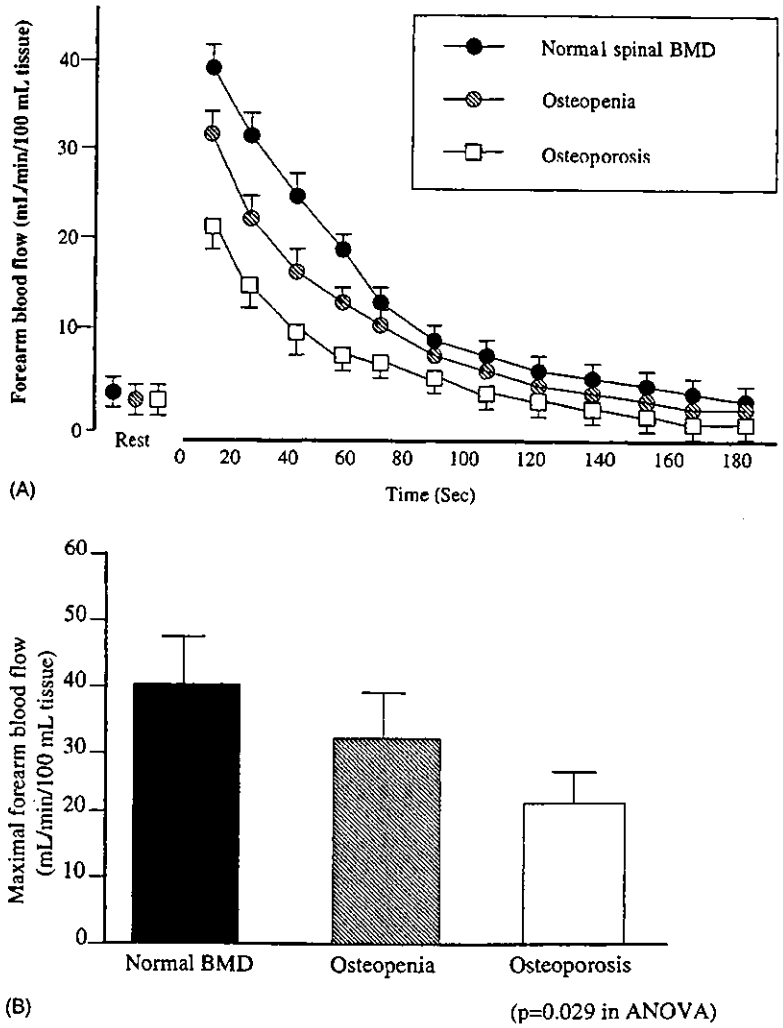

Fig. 1. Maximal FBF during reactive hyperemia in postmenopausal woman with normal spinal BMD, postmenopausal woman with osteopenia, and postmenopausal woman with osteoporosis (A). The maximal FBF was lower in the osteoporosis group than in the normal BMD and osteopenia groups (B: $P = 0.029$ by ANOVA). Results are reported as the mean ± S.D.

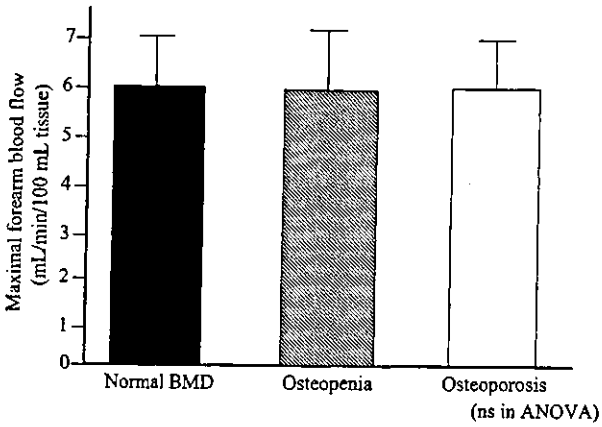

Fig. 2. Maximal FBF after the sublingual administration of NTG in the normal spinal BMD, osteopenia, and osteoporosis groups. There were no significant differences in the increase in FBF after treatment with sublingual NTG in the three groups. Results are reported as the mean ± S.D.

2.4. Statistical analysis

The results are presented as the mean ± S.D. One-way analysis of variance (ANOVA) was used to compare the clinical characteristics between the three groups (Table 1). Laboratory data for three groups were compared using one-way ANOVA with adjustment for age, body mass index, and years since the onset of menopause. The comparison of the time course for changes in FBF during reactive hyperemia between the three groups was analyzed by two-way ANOVA with repeated measures for one factor followed by the Bonferroni correction for multiple-paired comparisons. The repeated factor was time of reactive hyperemia and the nonrepeated factor was patient group (Fig. 1). Comparisons of variables, including maximal FBF response to nitroglycerine (Fig. 2), were performed using one-way ANOVA followed by the Bonferroni correction. Statistical analysis was performed with SPSS software (v8.0, Michigan, IL). A value for $P < 0.05$ was considered statistically significant.

3. Results

Table 1 summarizes the characteristics of the groups. Age and years since the onset of menopause were significantly greater in the osteoporotic group ($P < 0.001$ for both). Body weight and height were significantly lower in the osteoporotic group ($P < 0.001$ for both). After adjusting for age, body mass index, and years since the onset of menopause, there were no significant differences in serum estradiol, FSH, lipid, lipoprotein and apolipoprotein concentrations, or blood pressure and heart rate. However, a significant increase in the serum ACE activity was found in the osteoporotic group (14.7 ± 4.1 IU/L) compared to the normal BMD (11.4 ± 3.7 IU/L) and osteopenia (12.1 ± 3.6 IU/L) groups ($P = 0.042$ by ANOVA). In addition, significant decreases in the serum concentration of nitrite/nitrate were found in the osteoporotic group (23.2 ± 9.7 μM) than in patients with a normal BMD (38.0 ± 8.8 μM) or osteopenia (28.5 ± 7.3 μM) ($P = 0.041$ by ANOVA; Table 2).

In a further analysis of covariance, adjusted for age, body mass index, years since the onset of menopause, and basal FBF, women with osteoporosis had a lower maximal FBF response to reactive hyperemia (28.4 ± 8.8 mL/min per 100 mL tissue) than those with normal BMD (39.8 ± 6.8 mL/min per 100 mL tissue) or osteopenia (35.6 ± 7.5 mL/min per 100 mL tissue) ($P = 0.029$ by ANOVA; Fig. 1). However, there were no significant differences in the increase in the FBF after treatment with sublingually NTG in the three groups (Fig. 2).

4. Discussion

The most important finding of this study is the association between forearm endothelial function and bone mineral loss after adjusting for age, body mass index, and years since the onset of menopause, which supports a link between osteoporosis and cardiovascular disease in postmenopausal women. It has been shown that a low bone

Table 2
Clinical and laboratory characteristics of the three study groups

	Normal BMD	Osteopenia	Osteoporosis	*P*-value
Number	52	36	22	
Estradiol (pg/mL)	14.5 ± 5.2	13.1 ± 4.8	12.9 ± 4.3	0.399
FSH (IU/L)	71.2 ± 15.5	66.7 ± 15.8	63.3 ± 16.7	0.328
HDL cholesterol (mg/dL)	74.3 ± 12.6	67.7 ± 12.9	75.8 ± 14.4	0.136
LDL cholesterol (mg/dL)	139.7 ± 20.1	148.5 ± 24.8	157.8 ± 32.3	0.411
Triglyceride (mg/dL)	118.1 ± 38.3	117.3 ± 34.8	96.2 ± 35.6	0.703
Apolipoprotein AI (mg/dL)	163.9 ± 23.6	152.3 ± 24.1	161.4 ± 26.0	0.097
Apolipoprotein B (mg/dL)	102.4 ± 14.2	109.3 ± 14.8	116.4 ± 17.0	0.269
Systolic blood pressure (mmHg)	127.0 ± 13.0	124.5 ± 13.4	129.9 ± 15.1	0.648
Diastolic blood pressure (mmHg)	77.9 ± 7.8	75.0 ± 8.0	75.7 ± 10.2	0.549
Heart rate (bpm)	64.0 ± 1.2	64.5 ± 1.4	66.5 ± 2.1	0.587
ACE activity (IU/L)	11.4 ± 3.7	12.1 ± 3.6	14.7 ± 4.1	0.042
Nitrite/nitrate (μM)	38.0 ± 8.8	28.5 ± 7.3	23.2 ± 9.7	0.041

BMD, bone mineral density; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACE, angiotensin-converting enzyme. All results are presented as mean ± S.D. Analysis of covariance, adjusted for age, body mass index, and years since menopause.

mass in the elderly is associated with an increased rate of overall mortality, even if deaths following osteoporotic fractures are not considered. Recently, von der Recke et al. [3] reported that low bone mineral content at the onset of menopause is a risk factor for increased mortality in later life, especially from cardiovascular disease. The Rotterdam Study [17] found an association between low BMD and peripheral arterial disease in women but not in men. Barengolts et al. [18] reported that women with osteoporosis have a higher risk of developing coronary atherosclerosis using coronary calcium scores. Our present results support these findings and suggest that early postmenopausal women with low BMD, especially with osteoporosis, have impaired endothelial function in forearm resistance arteries. Furthermore, women with osteopenia might have been impaired endothelial function compared with women with normal BMD.

Because both osteoporosis and cardiovascular disease share major risk factors, such as decreased physical activity, hypertension, diabetes mellitus, and smoking, it is important to study these common etiological factors, which may partly explain the relationship between these two major diseases.

Indeed, Vogt et al. [10] found that after adjusting for age, BMD decreased with a decreasing ankle–arm index, a parameter of peripheral arterial disease, in elderly women. However, after adjusting for smoking and body mass index, this relationship disappeared. While the present study showed that after adjusting for age, years since the onset of menopause, and body mass index, a significant association was found between low bone mass and endothelial dysfunction in early postmenopausal women without a history of smoking or diabetes mellitus.

It is possible that there is not just one factor that is responsible for association between BMD and endothelial function. Estrogen is the best candidate that may be responsible for the relationship between a low BMD and endothelial dysfunction. Postmenopausal women with low circulating concentrations of endogenous estrogen have a lower bone mass [19]. Furthermore, an association between endogenous estrogen and endothelial function has been suggested [20]. Indeed, hormonal therapy in postmenopausal women increases BMD [21,22] and augments endothelial dysfunction [23,24]. Although estrogen may be one of important factors explaining the relationship between bone mass and endothelial function, there are several other possible explanations.

Reactive hyperemia in the peripheral arteries is mediated mainly by the release of nitric oxide (NO), an endothelium-derived relaxing factor [25]. NO is a free radical involved in the regulation of many physiologic processes, such as vascular relaxation [26], neurotransmission, platelet aggregation and immune responses [27]. It has become apparent that NO has important effects on bone cell function. Ralston et al. [28] reported that low concentrations of NO have been shown to potentate interleukin-1 induced bone resorption, based on the observation that NO synthase inhibitors inhibit interleukin-1 induced bone resorption in vitro. In the present study, we found that serum concentrations of nitrite/nitrate were significantly lower in osteoporotic women than in the other groups after adjustment for age, body mass index, and years since the onset of menopause. NO may play an important role in the pathologenesis both osteoporosis and endothelial dysfunction. In addition, the serum ACE activity was significantly higher in osteoporotic women than in the other group. ACE is important in cardiovascular homeostasis because this protease converts angiotensin I to angiotensin II and also degrades bradykinin. Increased ACE activity decreases the half-life of bradykinin and increases the production of angiotensin II [29]. Angiotensin II increases vascular superoxide production through activation of menbrane-associated nicotinamide adenine dinucleotide (NAD) diaphorase/nicotinamide adenine dinucleotide phosphate (NADP) diaphorase oxidase [30]. In a recent in vivo study, oxidative stress was found to inhibit osteoblastic differentiation of bone cells and to promote bone resorption through the recruitment and differentiation of osteoclast precursor cells [31,32].

There are other possible mechanisms responsible for our findings. Two bone associated proteins, osteopontin and matrix Gla protein, are expressed in atherosclerotic lesions [33,34]. Parathyroid hormone-related peptide, first identified as the factor responsible for malignancy-associated hypercalcemia, is a primary regulator of vascular tone [35].

4.1. Study limitations

Reactive hyperemia may be mediated by several factors, including NO, prostaglandin, endothelium-derived hyperpolarizing factor, and ischemia-induced adenosine release. In our previous study [36], the NO synthase inhibitor L-NMMA reduced reactive hyperemia by approximately 50% in Japanese patients. These findings suggest that NO may contribute to the ~50% of the FBF response to reactive hyperemia. This study could have been strengthened by increasing the sample size, since it appears that only 110 women were examined. Therefore, our present results need to be viewed with caution, even after adjusting for patients variables. Further studies are needed to determine the relationship between FBF responses to reactive hyperemia and bone mass or bone metabolism in postmenopausal women.

In conclusion, early postmenopausal women with low BMD, especially with osteoporosis, have endothelial dysfunction in forearm resistance arteries. These findings suggest that strategies for the prevention of osteoporosis should prevent endothelial dysfunction as well.

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Exercise and endothelial function: Role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients

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Abstract

Recent epidemiologic studies have shown that aerobic exercise, one of lifestyle modifications, reduces cardiovascular morbidity and mortality in the general population. However, the mechanisms underlying the anti-atherogenic and anti-hypertensive effects of exercise remain unclear. Hypertension is associated with alteration in endothelial function mediated through reduced nitric oxide (NO) bioavailability. Endothelial dysfunction is an early feature of atherosclerosis and vascular diseases in humans. Exercise training has been shown to improve endothelial function in animal models of hypertension and in patients with essential hypertension. These findings suggest that endothelial dysfunction in hypertension is reversible. Lifestyle modifications including exercise are expected to prevent cardiovascular complications through an augmentation of endothelial function in hypertensive patients. It is thought that exercise increases NO production and decreases NO inactivation, leading to an increase in NO bioavailability. In this review, we will focus on recent findings and on possible mechanisms underlying the beneficial effects of exercise on endothelial function in patients with hypertension.

Keywords: Aerobic exercise; Endothelial function; Endothelial nitric oxide synthase; Nitric oxide; Oxidative stress; Hypertension

Abbreviations: ACh, acetylcholine; Ang II, angiotensin II; cGMP, cyclic guanosine monophosphate; EDHF, endothelium-derived hyperpolarizing factor; eNOS, endothelial nitric oxide synthase; ET-1, endothelin 1; FGF, fibroblast growth factor; GPx, glutathione peroxidase; HIF-1, hypoxia-inducible factor-1; HSP, heat shock proteins; L-NMMA, N^G-monomethyl-L-arginine; NADH/NADPH, nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; PI3K, phosphatidyl-inositol-3-kinase; ROS, reactive oxygen species; SHR, spontaneously hypertensive rats; SOD, superoxide dismutase; VEGF, vascular endothelial growth factor; $V_{O_{2max}}$, maximum oxygen consumption.

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1. Introduction

Several investigators have observed that exercise training improves endothelial function in animal models of hypertension and in patients with hypertension (see references in Table 1). Regular physical exercise is associated with beneficial changes in blood pressure, lipid metabolism, glucose metabolism, neurohormonal factors, body weight, and shear stress (Martin et al., 1990; Wood et al., 1991; Arakawa, 1993; Paffenbarger et al., 1993). Although the mechanism of improvement in endothelial function during exercise has not been fully clarified, it is thought that regular aerobic exercise increases nitric oxide (NO) production with up-regulation of endothelial NO synthase (eNOS) gene expression and vascular endothelial growth factor (VEGF)-induced angiogenesis and decreases NO inactivation with augmented antioxidant system, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), and attenuation of nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidase activity, leading to an increase in NO bioavailability.

Table 1
Effects of exercise on endothelial function in hypertensive animals and humans

Animal/ human	Organ/tissue	Duration (weeks)	Endothelial function	Reference
SHR	plasma	10	NO_x ↑	Kohno et al., 2002
SHR	plasma	1–5	NO_x ↑	Jonsdottrir et al., 1998
SHR	femoral artery	single bout	phenylephrine response ↓	Rao et al., 2002
SHR	hypothalamus	9	NOS ↑	DiCarlo et al., 2002
SHR	hindlimb vascular	8–11	ACh response ↑	Chen et al., 1999
SHR	thoracic aorta (ring)	10	ACh response ↑	Chen et al., 1996a
SHR	thoracic aorta/ carotid artery (ring)	10	phenylephrine response ↓	Chen & Chiang, 1996b
SHR	thoracic aorta/ mesentric artery (ring)	12	ACh response ↑	Yen et al., 1995
SHRSP	plasma	8	NO_x ↑	Noguchi et al., 1999
Zucker rats	mesentric/ carotid artery (ring)	22	ACh response ↑	Arvola et al., 1999
WKY with L-NAME	plasma/ gastrocnemius muscle	10	NO_x ↑ / NOS ↑	Kuru et al., 2002
Hypertensive patients	forearm artery	12	reactive hyperemia ↑	Higashi et al., 1999a
Hypertensive patients	forearm artery	12	ACh response ↑	Higashi et al., 1999b

SHR, spontaneously hypertensive rats; NO_x, nitrate/nitrite; NOS, nitric oxide synthase; SHRSP, stroke-prone spontaneously hypertensive rats; WKY, Wistar Kyoto rats; L-NAME, *N*-nitro-L-arginine methyl ester.

2. Epidemiologic studies on exercise

Several nonpharmacological interventions are recommended for primary prevention of hypertension and other cardiovascular diseases (Castelli, 1984; Paffenbarger et al., 1993). Regular moderate physical exercise, such as walking, jogging, cycling, or swimming, being one of these interventions, lowers blood pressure in patients with mild essential hypertension. Regular aerobic exercise of moderate intensity decreases systolic blood pressure by 6–10 mm Hg and diastolic pressure by 4–8 mm Hg in patients with essential hypertension (Arakawa, 1993; Fagard, 2001). Epidemiologic studies have demonstrated that daily physical aerobic exercise prevents the cardiovascular mortality and morbidity (Castelli, 1984; Paffenbarger et al., 1993). Physical inactivity (sedentary state) per se is a risk factor for cardiovascular diseases. Subjects with low levels of physical fitness had a relative risk of 1.52 for development of hypertension when compared with subjects with high levels of fitness (Blair et al., 1984). The World Health Organization/International Society of Hypertension (1999) and the seventh report of the Joint National Committee of High Blood Pressure (Chobanian et al., 2003) recommend exercise at an intensity of ~ 50% of maximum oxygen consumption ($V_{O_{2max}}$), for 30 min per time, and 5–7 times per week, for patients with mild to moderate essential hypertension. According to these guidelines, the beneficial effects of exercise appear after 10 weeks when patients perform exercise for at least 30 min per time and at least 3 times per week.

It is clinically important to select the appropriate intensity, duration, frequency, and kind of exercise, because intense exercise can be hazardous to human vessels (Abraham et al., 1997; Bergholm et al., 1999). This moderate-intensity exercise fits the index of exercise training that is recommended from the preventive general viewpoint of cardiovascular diseases.

3. Endothelial function in hypertension

NO plays an important role in the regulation of vascular tone. In hypertensive patients, endothelium-dependent vascular relaxation in coronary, forearm, and renal arteries was found to be impaired, and endothelial dysfunction, which is involved in the development of atherosclerosis, was found to increase the risk of cardiovascular and cerebrovascular diseases (Drexler & Horning, 1999; Cai & Harrison, 2000). A great number of studies have shown that hypertension is associated with endothelial dysfunction (Panza et al., 1990, 1993; Treasure et al., 1993; Raij, 1993; Higashi et al., 1995, 1999b; Taddei et al., 1998). However, the mechanism underlying the impairment of endothelium-dependent vasodilation in hypertensive patients is unclear. Initially, agonists bind receptors and/or shear stress activates eNOS, and NO, which is produced from L-arginine in the presence of eNOS

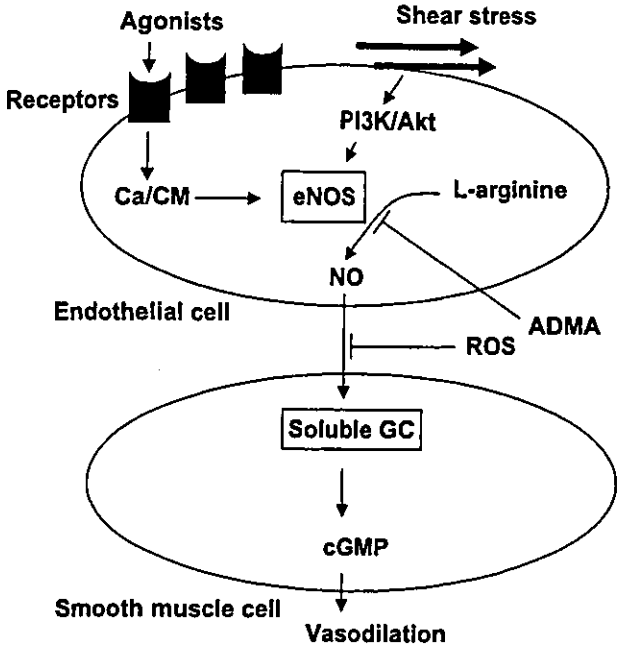

Fig. 1. Putative mechanisms of impairment of endothelial function in hypertension. It is thought that various factors contribute to the impairment of endothelium-dependent vasodilation in hypertension, but the precise mechanisms remain unclear. The possible mechanisms that are pointed out are shown with underlines; increase in the amount of the endogenous eNOS inhibitor asymmetrical dimethylarginine (ADMA), increase in vasoconstrictors, such as angiotensin II, endothelin-1, and norepinephrine, and inactivation of NO by ROS.

in the endothelium, activates cytosolic guanylate cyclase and increases cyclic guanosine monophosphate (cGMP) content in vascular smooth muscle cells, resulting in relaxation of vascular tone.

Thus, it seems reasonable to assume that there is a trouble somewhere in this L-arginine-NO-cGMP pathway (Fig. 1).

4. Exercise and endothelial function

Recent experimental studies have demonstrated that continued exercise augmented vasodilation evoked by the endothelium-dependent vasodilator acetylcholine (ACh) in dogs (Sessa et al., 1994) and rats (see references in Table 1). We found that physical training enhanced endothelium-dependent vasodilation in forearm circulation in hypertensive patients (Higashi et al., 1999a, 1999b) as well as in healthy individuals (Higashi et al., 1999b; Goto et al., 2003). A 12-week moderate intensity exercise program improved endothelium-dependent vasodilation with ACh, but not endothelium-independent vasodilation with isosorbide dinitrate (Higashi et al., 1999a, 1999b; Goto et al., 2003). These findings indicate that the augmentation of ACh-induced vasorelaxation may be related to an improvement in the function of the endothelium, but not vascular smooth muscle. Recently, we reported that long-term moderate intensity (50% $V_{O_{2max}}$) exercise, but not mild (25% $V_{O_{2max}}$) or high-intensity (75% $V_{O_{2max}}$) exercise, augmented endothelium-dependent vasodilation in healthy subjects (Goto et al., 2003). This moderate-intensity exercise fits the index of exercise training that is recommended from the general viewpoint of prevention of cardiovascular diseases. Effects of exercise on endothelial function in hypertensive animals and human are summarized in Table 1. All results from the 13 studies show that either acute or chronic exercise has beneficial effects on endothelial function in different types of species, organ, or tissues in hypertension (see references in Table 1). A large number of studies have shown that even in normal control animals (Wang et al., 1993; Sessa et al., 1994; Bernstein et al., 1996) and healthy subjects (Green et al., 1994; Kingwell et al., 1997), exercise training augments endothelial function.

Exercise training augments endothelial function in animal models of hypertension and in patients with essential hypertension as well as in healthy individuals. The long-term moderate intensity exercise, but not mild or high-intensity exercise, augments endothelium-dependent vasodilation in healthy subjects and probably in hypertensive patients.

5. Increase in nitric oxide production

There are several possible explanations for the augmentation of endothelial function by regular aerobic exercise in patients with essential hypertension. Although the anti-hypertensive and anti-atherogenic mechanisms of exercise have not been clarified, one possible mechanism of the beneficial effect of exercise is improvement in endothelial function through an increase in NO bioavailability (increase in NO production and/or decrease in NO inactivation).

5.1. Endothelial nitric oxide synthase

One possible mechanism by which long-term aerobic exercise augments endothelial function is an increase in vascular shear stress resulting from increased blood flow. Acute or chronic increases in shear stress potently stimulate the release of NO in isolated vessels (Miller & Vanhoutte, 1988) and cultured cells (Uematsu et al., 1995). Sessa et al. (1994) have demonstrated that the increase in shear stress in epicardial coronary arteries of dogs for 10 days of treadmill exercise enhanced the expression of the vascular eNOS gene leading to ACh-stimulated NO release. The up-regulation of eNOS mRNA levels and eNOS protein levels during exercise training may contribute to improvement in endothelial function through an increase in NO production.

Exercise training, probably by an increase in shear stress, exerts its beneficial effects on endothelial function by activation of several signal transduction pathways (Traub & Berk, 1998). It is thought that mechanosensors, such as caveolae (Garcia-Cardena et al., 1998), G-proteins (Tseng et al., 1995), ion channels (Schwartz & Lechene, 1992), and

integrins (Muller et al., 1997) on the membranes of endothelial cells sense shear stress and transduce stimuli into biochemical signals, and then several stimuli activate Ras/Raf/MEK/ERK (Traub & Berk, 1998) and c-Src (Davis et al., 2003a, 2003b) pathways, leading to an increase in eNOS activity.

The response of endothelial cells to shear stress activates tyrosine kinase c-Src (Traub & Berk, 1998; Davis et al., 2001). Davis et al. (2003a) showed that c-Src plays an important role in the modulation of eNOS gene expression during exercise training. They postulated 2 pathways of c-Src-induced eNOS gene expression in response to exercise: increased transcription of eNOS by activation of the Ras/Raf/MEK/ERK pathway and prolonged message stabilization by an unidentified signal pathway. In addition, c-Src increases extracellular SOD, which is a scavenger of reactive oxygen species (ROS), in an increase in eNOS-dependent manner in response to shear stress (Davis et al., 2003a). c-Src may be one of key intracellular signaling molecules during exercise training. Recently, Davis et al. (2003b) revealed one of the nuclear events that lead to an increase in eNOS transcription in response to shear stress. Shear stress increases eNOS transcription by nuclear factor κB activation and p50/p65 binding to a GAGACC sequence present in the human eNOS promoter (Davis et al., 2003b).

The stimulation of cells, including endothelial cells, by shear stress can lead to the phosphorylation and activation of Akt (Dimmeler et al., 1999). Shear stress stimulates the phosphorylation of eNOS on its Akt-related phosphorylation site Ser^{1177} in humans and Ser^{1179} in bovines independently of an increase in intracellular calcium (Dimmeler et al., 1999). The phosphatidyl-inositol-3-kinase (PI3K) and Akt pathway, which causes intracellular calcium-independent eNOS phosphorylation and activation, is involved in a shear stress-activated signal transduction cascade.

Heat shock proteins (HSP) are present in most cells, including endothelial cells, and play an important role in normal cellular homeostasis and cell protection from damage in response to stress stimuli (Garcia-Cardena et al., 1998; Xu, 2002). Exercise is a physiological stimulus factor of HSP (Xu, 2002). Several investigators have focused on the interaction of eNOS with HSP90 (Garcia-Cardena et al., 1998; Russell et al., 2000). HSP90 up-regulates eNOS activity in endothelial cells by forming an eNOS-HSP90 heterocomplex in response to shear stress (Garcia-Cardena et al., 1998; Fleming & Busse, 1999; Russell et al., 2000). Although binding of HSP90 to Akt is necessary for Akt activation, Brouet et al. (2001) have recently shown that HSP90 stimulates eNOS activation independently of Akt phosphorylation on Ser^{1177}.

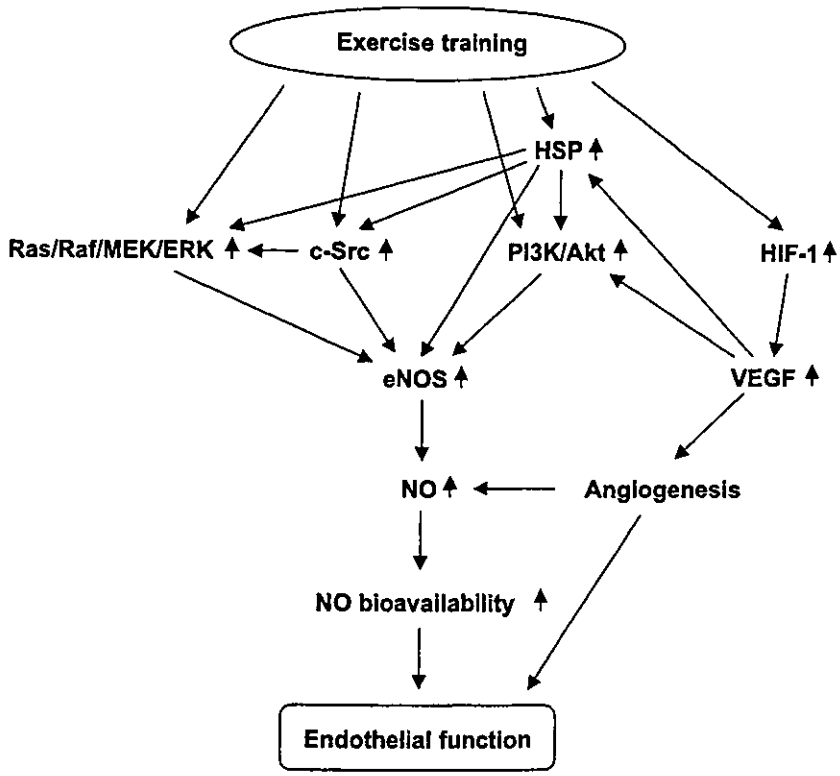

Fig. 2. Putative mechanisms by which exercise training improves endothelial function through an increase in NO bioavailability: an increase in NO production. Signal molecules of c-Src, Raf/Ras/MEK/ERK, and Akt, and these molecular chaperone HSP and HIF-1-induced VEGF lead to an increase in endothelial NO synthase (eNOS) activity, and VEGF-induced angiogenesis, resulting in an increase in NO production. An increase in NO production leads to an increase in NO bioavailability.

Putative shear stress-mediated mechanotranductions, such as the Ras/Raf/MEK/ERK pathway, c-Src, PI3K/Akt, HSP, and hypoxia-inducible factor-1 (HIF-1), may contribute to the up-regulation of eNOS mRNA and eNOS protein during exercise training, leading to an increase in NO production (Fig. 2). Shear stress is sensed and transduced into biochemical signals by multiple pathways in the vasculature, resulting in various biological responses, including increases in eNOS activity.

5.2. Vascular endothelial growth factor: angiogenesis

Regular aerobic exercise has been shown to lead to functional and histological alterations in the vascular endothelium, resulting in enhanced vascular structure and function (Niebauer & Cooke, 1996). Furthermore, exercise training increases capillary density and the capillary-to-fiber ratio in skeletal muscle in humans (Hudlicka et al., 1992). Various angiogenetic factors, such as VEGF and fibroblast growth factor (FGF), play an important role in angiogenesis in animals as well as in humans (Lee & Feldman, 1998). Several investigators have reported that acute exercise up-regulates VEGF mRNA and protein levels in skeletal muscle in animals (Olfert et al., 2001; Lloyd et al., 2003) and in humans (Gavin et al., 2003). Swimming increases circulating VEGF levels in humans (Asano et al., 1998). Lloyd et al. (2003) have shown that although angiogenesis is observed from day 12 of exercise training in rats, VEGF gene expression is detected during the initial phase of training program and is gradually decreased as the training progresses. These findings suggest that increased VEGF protein levels contribute to angiogenesis during the early phase of the training program. In addition, Fontana et al. (2002) reported that VEGF stimulates the recruitment of HSP90- and PI3K/Akt-dependent eNOS phosphorylation, leading to an increase in NO production.

Several lines of evidence have indicated that hypoxia per se enhances VEGF gene expression (Olfert et al., 2001; Gavin et al., 2003). It is well known that VEGF gene expression is up-regulated by HIF-1 under the condition of hypoxia (Gustafsson & Kraus, 2001). HIF-1 is a heterodimer composed of 2 subunits, HIF-1α and HIF-1β, and promotes transcription by combining with hypoxia response element in its target gene (Gustafsson & Kraus, 2001). Hypoxia up-regulates VEGF receptor Flt-1 gene expression in endothelial cells, while the expression levels of another VEGF receptor, the KDR gene, does not change (Gavin & Wagner, 2002). Since hypoxia response element is located in the promoter region of the Flt-1 gene, the location of hypoxia response element may be related to the hypoxia-induced Flt-1 gene expression (Gerber et al., 1997). Exercise induces hypoxia in skeletal muscle (Gustafsson & Kraus, 2001).

FGF also appears to be important in angiogenesis in skeletal muscle. Although Olfert et al. (2001) reported that normoxic exercise training increased basic FGF mRNA levels by about 2-fold in rat skeletal muscle, most studies have not shown a significant increase in basic FGF and FGF-2 mRNA levels after exercise training. Hypoxia, but not FGF-2 gene expression, induced VEGF gene expression in vitro and in vivo studies (Gustafsson & Kraus, 2001). It is unlikely that FGF plays a more important role than that of VEGF in angiogenesis in skeletal muscle during exercise.

The hypoxia-HIF-1-VEGF pathway may play an important role in exercise-induced angiogenesis in skeletal muscle (Fig. 2).

6. Decrease in nitric oxide inactivation (oxidative stress)

Several studies using in animal hypertensive models and human subjects with hypertension have shown that endothelial dysfunction is associated with an increase in ROS ((Dijhorst-Oei et al., 1999; Romero & Reckelhoff, 1999; Cai & Harrison, 2000)). Amount of antioxidant scavengers, such as SOD, glutathione, and vitamins C and E, are decreased in patients with hypertension (Irani, 2000). NADH/NADPH oxidase, which is a major source of production of ROS in vessel walls, is activated in hypertensive rats (Rajagopalan et al., 1996). It has also been shown that ascorbic acid (vitamin C) restores impaired endothelium-dependent vasodilation in patients with essential hypertension (Taddei et al., 1998). Therefore, enhanced production of ROS and an attenuated antioxidant system may contribute to endothelial dysfunction in hypertensive patients. In other words, enhanced NO inactivation caused by excess ROS production, rather than decreased NO production, may play an important role in impaired endothelium-dependent vasodilation in hypertension.

In healthy subjects, exercise of mild intensity did not alter any parameters, including oxidative stress and endothelial function (Goto et al., 2003). Interestingly, a 12-week period of exercise of high intensity increased the indices of oxidative stress, such as plasma concentration of 8-hydroxy-2′-deoxyguanosine and serum concentration of malondialdehyde-modified low-density lipoprotein and decreased endothelium-dependent vasodilation in healthy young men (Goto et al., 2003). It is thought that ROS are not produced excessively under physiological conditions in healthy subjects. Davies et al. (1982) reported that the massive increase in oxygen uptake that occurs in skeletal muscle during exercise is associated with an increase in the generation of ROS. These findings suggest that exercise of high-intensity increases oxidative stress. It was thought that increased oxidative stress induced by exercise of high intensity will diminish endothelium-dependent vasodilation. However, we did not find impaired endothelial function associated with increased oxidative stress in healthy subjects (Goto et al., 2003). Matsumoto et al. (1994) reported that the production of NO progressively increases as exercise intensity increases. Although we did not assess the production of NO, it is

possible that exercise of high-intensity increases NO production.

These findings suggest that a decrease in NO inactivation contributes to the improvement in endothelial function in patients with hypertension. The action of increased ROS that inactivates NO was removed by increased NO production, resulting in maintenance of endothelial function. Exercise of moderate intensity may predominately increase NO production compared with ROS production, leading to augmentation of endothelial function in healthy subjects.

6.1. Antioxidant system: Superoxide dismutase, glutathione peroxidase, and catalase

Protective antioxidant mechanisms are complex and multifactorial. Although exercise training increases ROS, exercise training works as a result in improving endothelial function. Antioxidant defense system, such as SOD, GPx, and catalase, scavenges ROS in the vasculature, resulting in inhibition of NO degradation. The susceptibility of vascular cells to oxidative stress is a function of the overall balance between the degree of oxidative stress and the antioxidant defense capability. The antioxidant enzyme SOD rapidly dismutates superoxide to hydrogen peroxide. SOD has been identified as 3 enzymatic types: Cu/Zn-SOD, Mn-SOD, and extracellular SOD. Steady laminar shear stress up-regulates the gene expression of Cu/Zn-SNO and Mn-SOD in endothelial cells (Inoue et al., 1996; Hollander et al., 1999). Exercise training enhances the protein level and enzymatic activity of SOD, such as Cu/Zn-SOD and Mn-SOD, in the vascular endothelium and smooth muscle cells of the aorta in various animal models (Yamashita et al., 1999; Rush et al., 2003). In the vasculature of humans, ~ 50% of total SOD is extracellular SOD (Stralin et al., 1995). Recently, Fukai et al. (2000) have demonstrated that exercise of moderate intensity for 3 weeks increased eNOS and extracellular SOD protein levels in wild-type mice but had no effect on extracellular SOD protein level in eNOS knockout mice and that the effect of endothelium-derived NO on extracellular SOD protein level is mediated by the cGMP/protein kinase G-dependent pathway.

Takeshita et al. (2000) reported that a physiological level of shear stress up-regulates GPx mRNA levels and GPx enzymatic activity in cultured bovine aortic endothelial cells. Many studies have shown adaptive changes in GPx and catalase gene expression in various tissues, such as skeletal muscle (Ji et al., 1992), myocardium (Somani & Rybak, 1996), and erythrocytes (Somani et al., 1995), in response to various types of exercise. However, none of those studies showed up-regulation of antioxidant enzymatic activity, including that of SOD, after exercise (Johnson, 2002; Urso & Clarkson, 2003). These discrepancies may be explained by the different exercise types, species, and targets of organ and tissues used in those studies.

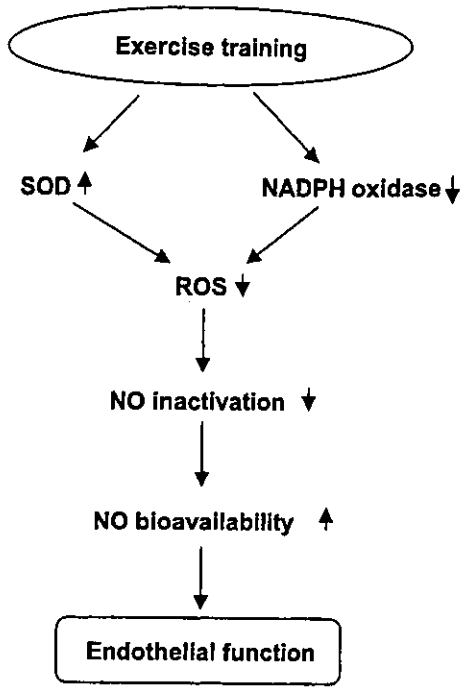

Fig. 3. Putative mechanisms by which exercise training improves endothelial function through an increase in NO bioavailability: a decrease in NO inactivation, an increase in SOD, and a decrease in NADH/NADPH oxidase activity are involved in the reduction in ROS, leading to a decrease in NO inactivation. A decrease in NO inactivation leads to an increase in NO bioavailability.

The up-regulation of Cu/Zn-SOD, Mn-SOD, extracellular SOD, GPx, and catalase induced by aerobic exercise may improve endothelial function through the inhibition of NO degradation with a decrease in ROS (Fig. 3). However, it is not clear at present whether exercise training alters the antioxidant defense system.

6.2. Nicotinamide adenine dinucleotide/ nicotinamide adenine dinucleotide phosphate oxidase

NADH/NADPH oxidase is the most important source of superoxide in the vasculature (Cai & Harrison, 2000; Sowers, 2002). It is thought that inactivation of NADH/NADPH oxidase may contribute to the improvement in endothelial function after aerobic exercise in patients with hypertension. Interestingly, Rush et al. (2003) have shown that exercise training for 16 to 19 weeks resulted in a decreases in the subunit of NADH/NADPH oxidase $p67^{phox}$, but not $p47^{phox}$, in the porcine aortic endothelium. However, the effects of exercise on other membrane-spanning components, such as $p22^{phox}$ and $gp91^{phox}$, and the cytosolic component Rac 1 were not elucidated in their study. Recently, Zalba et al. (2000) showed that endothelial dysfunction is due to an excess of ROS rather than a decrease in NO production in the aorta of spontaneously hypertensive rats (SHR) and is associated

with both the up-regulation of $p22^{phox}$ mRNA expression and the increased activity of NADH/NADPH oxidase.

These findings suggest that aerobic exercise may improve endothelial function through a decrease in ROS production with inactivation of NADH/NADPH oxidase (Fig. 3). Further studies on the mechanisms underlying the effects of exercise on the components of NADH/NADPH oxidase in humans are awaited for future therapeutic benefits.

6.3. Mechanical pressure (hypertension)

Endothelial function becomes progressively more impaired as blood pressure increases, and the degree of dysfunction is related to the severity of hypertension (Dohi et al., 1990; Panza et al., 1993). Therefore, it is expected that endothelial dysfunction will be improved by lowering blood pressure. Although clinically effective anti-hypertensive therapies, such as angiotensin converting enzyme inhibitors and aerobic exercise, have restored resistance artery endothelial function of forearm circulation in patients with essential hypertension, there is no correlation between degree of reduction in blood pressure and augmentation of endothelium-dependent vasodilation (Schiffrin & Deng, 1995; Higashi et al., 2000). In addition, regular aerobic exercise does not alter blood pressure in normotensive subjects (Higashi et al., 1999b, Goto et al., 2003), while exercise improves endothelial function in normotensive subjects. Therefore, a reduction in blood pressure per se may not be involved in the restoration of resistance artery endothelial function in forearm circulation. However, we should carefully interpret data on the effects of hypertension (mechanical pressure) on endothelial function. Several lines of evidence have indicated the mechanical pressure-induced activation of NADH/NADPH oxidase, which generates ROS (Sowers, 2002).

These finding suggest that mechanical pressure per se impairs endothelial function through the inactivation of NO. Furthermore, removal of mechanical pressure may restore endothelial function in hypertension. However, it is not known whether exercise-induced reduction in blood pressure directly contributes to the improvement in endothelial function and increase in NO production.

6.4. Vasoconstrictors

A balance of vasodilators and vasoconstrictors also plays an important role in the physiologic regulation of vascular tone (Lüscher, 1990). Angiotensin II (Ang II)-induced NADH/NADPH oxidase activation is one of the major sources of superoxide in hypertension (Rajagopalan et al., 1996; Romero & Reckelhoff, 1999; Higashi et al., 2002a). Recently, we have shown that plasma Ang II levels do not alter during aerobic exercise of mild, moderate, or high intensity in healthy young men or during exercise of moderate intensity in hypertensive patients (Higashi et al., 1999b; Goto et al., 2003). It is unclear whether reduction in local Ang II levels contributes to exercise-induced improvement in endothelial function in hypertension. It is unlikely that Ang II plays a critical role in augmentation of endothelial function during exercise training in healthy subjects who do not have an activated renin-angiotensin system.

Maeda et al. (2001) reported that chronic aerobic exercise decreases plasma endothelin 1 (ET-1) concentrations even in healthy young humans. Recent data have shown that there is no effect of exercise on plasma ET-1 concentrations (Lavrencic et al., 2000). We also did not find a significant change in plasma ET-1 concentrations during aerobic exercise of mild, moderate, or high intensity in healthy young men or during exercise of moderate intensity in hypertensive patients (Higashi et al., 1999b). Therefore, evidence of reduction in circulating ET-1 levels with exercise training is not conclusive.

Although norepinephrine is not released from the vascular endothelium, it is a major vasoconstricting factor. We found that long-term aerobic exercise significantly reduced plasma norepinephrine concentration in patients with hypertension (Higashi et al., 1999a, 1999b). This finding is consistent with results of previous studies showing that exercise training decreases circulating norepinephrine levels and attenuates sympathetic nervous activation in animal models and in humans with hypertension (Mathias, 1991). Regular exercise may play an important role in protection of the endothelium through reduction in norepinephrine, leading to augmented ACh-stimulated NO release in hypertensive patients. However, plasma norepinephrine concentrations were similar before and after exercise of any intensity in healthy subjects, whereas moderate exercise, but not exercise of mild or high intensity, augmented endothelial function in healthy subjects (Goto et al., 2003). Therefore, the differences in vascular responses to ACh before and after exercise of moderate intensity cannot be explained by differences in sympathetic nervous system activity in healthy subjects.

Exercise training may augment endothelial function through a decrease in vasoconstrictors.

7. Prostaglandins and endothelium-derived hyperpolarizing factor

Other endothelium-dependent vasodilators, such as prostaglandins and endothelium-derived hyperpolarizing factor (EDHF), may also contribute to exercise-induced vasodilation. Griffin et al. (1999) showed that exercise training improves endothelium-dependent vasodilation in the coronary artery of the swine after chronic coronary occlusion through an increase in the production of NO and EDHF. Yen et al. (1995) reported that chronic exercise augments ACh-induced vasodilation in SHR through an increase in NO and EDHF, but not prostaglandins, production. In addition, the administration of prostaglandin synthesis inhibitors reduced exercise-induced vasodilation by only ~ 10% in humans,

suggesting that prostaglandins may play a minimal role in exercise-induced vasodilation (Willson & Kapoor, 1993), although it is well known that shear stress stimulates secretion of prostacyclin from endothelial cells.

Results of further studies on the effects of prostaglandins and EDHF on vascular function during exercise will enable more specific conclusions regarding the role of aerobic exercise in endothelium-dependent vasodilation in humans to be drawn.

8. Pharmacological therapeutic implications

Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis, resulting in cardiovascular complications (Ross, 1999). From a clinical perspective, it is important to select an appropriate intervention that is effective in improving endothelial function in hypertensive patients. Several interventions, including treatment with anti-hypertensive agents, such as angiotensin-converting enzyme inhibitors (Schiffrin & Deng, 1995; Higashi et al., 2000), supplementation therapy, such as a substrate of NO L-arginine (Higashi et al., 1995), a cofactor of NO tetrahydrobiopterine (Higashi et al., 2002b), treatment with antioxidants vitamin C (Taddei et al., 1998); and lifestyle modifications, such as aerobic exercise (Higashi et al., 1999a, 1999b), body weight reduction (Sasaki et al., 2002), and sodium restriction (Bragulat et al., 2001) have been shown to improve endothelial function and prevent cardiovascular complications in patients with essential hypertension.

The results of a series of studies on the effects of exercise on endothelial function have shown that more specific antioxidative agents are required.

9. Conclusions

Beneficial effects of exercise, such as lowered lipoprotein level, increased shear stress, reduced vasoconstrictors, and lowered blood pressure, may independently or interdependently contribute to improvement in endothelial function through increase in NO release and/or inhibition of NO degradation. In healthy subjects, it is likely that shear stress-induced increase in eNOS activity predominantly contributes to the augmentation of endothelial function during exercise training. In hypertensive patients, both increased NO production and decreased NO inactivation may have an influence exquisitely and beneficial effects of exercise are expressed.

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