

Endothelial Dysfunction and Alteration of Nitric Oxide/Cyclic GMP Pathway in Patients with Exercise-Induced Hypertension

Hyuk-Jae Chang, Jaehoon Chung, Byoung-Joo Choi, Tae-Young Choi, So-Yeon Choi, Myeong-Ho Yoon, Gyo-Seung Hwang, Joon-Han Shin, Seung-Jea Tahk, and Byung-il William Choi

Department of Cardiology, Ajou University School of Medicine, Suwon, Korea.

The diagnostic and prognostic implication of exaggerated blood pressure response to exercise have been controversial, with opinions ranging from a benign process to a harbinger of potential cardiovascular morbidity. Endothelial dysfunction has been demonstrated in patients with atherosclerosis and as a risk factor for coronary artery disease. However, whether the cause of exercise-induced hypertension might be related to endothelial dysfunction has not been well elucidated. We evaluated endothelial function in patients who showed a systolic blood pressure ≥ 210 mmHg in males and ≥ 190 mmHg in females during treadmill exercise test. We measured the endothelial function of the brachial artery in 35 patients with exercise-induced hypertension, and in 35 age- and gender-matched normal control subjects, by a high resolution ultrasound technique, and the concentration of $\text{NO}_2^-/\text{NO}_3^-$ and cyclic guanosine monophosphate (GMP). Endothelial-dependent vasodilation was impaired in patients with hypertension compared to normal controls (3.14 ± 0.61 vs. $6.5 \pm 0.76\%$, $p < 0.05$). The extent of vasodilation was significantly correlated with age ($r = -0.28$, $p < 0.05$) and systolic blood pressure difference ($r = -0.36$, $p < 0.05$). The levels of $\text{NO}_2^-/\text{NO}_3^-$ and cyclic GMP at maximal exercise were significantly higher than those at rest and recovery in both controls and the hypertensive group ($p < 0.05$). Although there was no significant difference in the increment of $\text{NO}_2^-/\text{NO}_3^-$ during maximal exercise between the controls and hypertensive group (55 ± 17 vs. $56 \pm 12 \mu\text{mol/L}$, $p = \text{NS}$), cyclic GMP level during maximal exercise was significantly higher in the control group than the hypertensive group (10 ± 1.8 vs. $8.3 \pm 2.5 \text{ pmol/ml}$, $p < 0.05$). Patients with exercise-induced hypertension have poor endothelium-dependent vasodilation due to an impaired nitric oxide/cyclic GMP pathway, which may play a significant role

in increasing blood pressure during exercise with inadequate peripheral adjustment to changing cardiac output.

Key Words: Exercise test, hypertension, endothelium, nitric oxide, cyclic GMP

INTRODUCTION

The exaggerated increase of systolic blood pressure during exercise has been often found during treadmill stress testing, not only in patients with systemic hypertension but also those with normal blood pressure at rest. The exact mechanism and prognostic implication have been unclear and conflicting results have often been reported. However, in many epidemiological studies, exercise-induced hypertension has been implicated to predict future cardiovascular events such as systemic hypertension, congestive heart failure and ischemic heart diseases.¹⁻³

Although diastolic blood pressure may not increase or often decrease during exercise tests, systolic blood pressure has been increasing at a steady rate, as a function of the increase in cardiac output against peripheral adaptation during exercise.⁴ Nitric oxide (NO) from the vascular endothelium has been implicated to relax vascular smooth muscle, thereby decreasing vascular resistance and blood pressure.⁵⁻⁷

Since Celermajer, et al.⁸ had established a non-invasive measurement of endothelial function by ultrasound, endothelial function has been evaluated for the prognosis and response to treatment as an independent predictor of future cardiovas-

Received July 8, 2003
Accepted August 18, 2003

Reprint address: requests to Dr. Hyuk-Jae Chang, Department of Cardiology, Ajou University School of Medicine, San 5 Wonchon-dong, Paldal-gu, Suwon 442-721, Korea. Tel: 82-31-219-5712, Fax: 82-31-219-5708, E-mail: hjchang70@hotmail.com

cular events.^{9,10} NO from the vascular endothelium may act through an increase in cyclic GMP. Nitrite and nitrate, metabolites of NO, have been implicated to reflect the amount of NO production in the vascular system.¹¹

In this study, the pathogenesis of exercise-induced hypertension was evaluated by ultrasound, and the serum concentration of nitrite/nitrate and cyclic GMP was measured prior to, during and after treadmill exercise, to define the mechanism and clinical implications of exercise-induced hypertension.

MATERIALS AND METHODS

Patient population

Consecutive 35 patients from July 2000, who were admitted to Ajou University Hospital for health screening and who were documented to have normal blood pressure at rest but exaggerated systolic blood pressure response during treadmill exercise test (≥ 210 mmHg in males and ≥ 190 mmHg in females) were enrolled along with 35 age- and gender-matched control subjects who showed normal systolic blood pressure response during exercise.^{12,13}

Study subjects were excluded if they met any of the following criteria: (1) hypertension, defined as the current use of antihypertensive medication or a resting blood pressure of ≥ 140 mmHg systole or 90 mmHg diastole at physical examination; (2) a history or clinical evidence of coronary heart disease, congestive heart failure, or valvular or congenital heart disease; (3) use of cardiac medication; (4) age > 60 ; (5) any chronic disease of the liver, lungs or kidneys, or diabetes mellitus.

We reviewed the patients' histories and performed physical examinations with laboratory tests including biochemical analysis, chest PA and electrocardiogram (EKG), and measured cardiac function and left ventricular mass index by echocardiography according to the method of Deveux, et al.¹⁴

All patients were studied with a multistage exercise treadmill test according to Bruce protocol. Blood pressure, heart rate and a 12-lead EKG were recorded prior to exercise, during the last minute

of each 3-minute exercise stage and after 5 minutes after exercise during the recovery phase. All subjects gave the informed consent and the protocol of study was approved by the institutional committee of medical ethics.

Measurement of the endothelial function by ultrasound⁸⁻¹⁰

The diameter of the brachial artery was measured within 1 week of the treadmill exercise test by a B mode ultrasound, 10.0 MHz trapezoidal linear array transducer (axial resolution, 0.12 mm; penetration depth, 2-16 cm) with HP SONOS 5500 imaging system (Hewlett-Packard, Andover, Massachusetts, USA). All procedures were recorded with super-VHS videocassette recorder (AG-MD 830, Panasonic, Tokyo, Japan) and the images of the brachial artery were magnified 8 times and printed out through a Sony videographic printer (UP 5600MDU). All patients were kept in NPO (nothing by mouth) for 12 hours before measurement.

Vessel diameter was measured by two observers, who were unaware of the clinical details and the stage of the experiment. The arterial diameter was measured at a fixed distance from an anatomical marker, such as a bifurcation, with ultrasonic calipers. Measurements were taken from the anterior to posterior "m" line, the interface between media and adventitia, at end-diastole gated on the EKG R wave. For the reactive hyperemia scan, diameter measurements were taken 45-60 seconds after cuff deflation. Four cardiac cycles were analyzed for each scan and the measurements were averaged.

In each patient, scans were taken at rest, during reactive hyperemia, again at rest, and after sublingual nitroglycerin. The subjects were placed in the supine position for at least 10 minutes before the first resting scan was recorded. Increased flow was then induced by inflation of a pneumatic tourniquet to a pressure of 300 mmHg for 5 minutes. A second scan was taken 45-60 seconds after cuff deflation. Fifteen minutes was allowed for vascular recovery and consecutive resting scan was taken. Sublingual nitroglycerin (0.3 mg) was administered, and 3 minutes later the last scan was performed. Vessel diameters in scans after

reactive hyperemia, 15 minutes at rest, and with nitroglycerin were expressed in percentages of the first control scan, i.e. % diameter changes=(VD [during reactive hyperemia or after nitroglycerine]-VD[resting])/VD[resting], (VD: vessel diameter). The mean vessel diameter and percent dilatation for each patient were obtained by averaging the measurements taken during the procedures. For every patient, blood pressure was recorded in the opposite arm before the measurement. Resting vessel diameter in controls was measured 4 times repeatedly to find the intra-observer variability with coefficient variation of 2.1%. Two other researchers evaluated the same vessel diameter separately to determine inter-observer variability with a correlation coefficient of 0.901 which was similar with other studies.⁸

Measurement of cyclic GMP and NO₂⁻/NO₃⁻

After the measurement of the endothelial function by ultrasound, all patients undertook treadmill exercise test in the following day up to 60% of their maximal exercise capacity using the Bruce protocol. To attenuate the effect of the nitrogen contained in the diet, every patient fasted for 12 hours before the test. After the catheter (1.16 in, 20 gauge, Angiocath™ Plus, Becton Dickinson, NJ, USA) was inserted into the right brachial vein, blood was drawn from the patients at rest, maximal exercise, and 30 minutes after exercise for the measurement of cyclic GMP and NO₂⁻/NO₃⁻. Specimens were kept at -70°C after centrifugation (5000 rpm, 15 min at 4°C). To obtain the NO₂⁻/NO₃⁻ ratio, the plasma nitrite and nitrate levels were assayed via nitrite using the NO colorimetric assay based on the Griess reaction.¹¹ Nitrate was reduced to nitrite by adding nitrate reductase (*E. coli* [ATCC25922], American Type Collection, Rockville, MD, U.S.A.). Then, after deproteinization, we measured absorbance with a microplate reader Labsystem at 540 nm. We calculated the concentration of nitrite by applying the absorbance to the standard curve by sodium nitrate (BDH Chemical Co., Dorset, UK). The level of cyclic GMP was evaluated with a commercially available ELISA kit (c-GMP assay RPA 525; Amersham International, Buckinghamshire, UK).

Statistical analysis

Statistic analysis was performed using SPSS/PC software (SPSS for windows, Release 8.0.0) and all data are expressed as mean ± SD. Comparison between the two groups was performed with χ^2 -test for discrete variables and with Student's t-test or Mann-Whitney U test for continuous variables. Correlation and regression analyses were also performed. Statistical significance was accepted with $p < 0.05$.

RESULTS

Patient characteristics

Thirty-five patients with exercise-induced hypertension (group A) were compared with the 35 age- and gender-matched controls (group B). Subjects consisted of 66 men and 4 women with age ranging from 33 to 60 (mean 45.4 ± 8.1). There was no difference in the prevalence of cardiovascular risk factors, i.e. smoking history, dyslipidemia, family history of premature coronary artery disease, between the two groups. Also, there was no statistical difference in the fasting glucose and lipid profile known to affect the endothelial function between the two groups (Table 1). However, left ventricular hypertrophy on EKG was more frequent in group A (40%) than in group B (14%) ($p < 0.05$).

Left ventricular systolic dysfunction and significant heart disease were not noted on the echocardiography in either groups. However, the left ventricular mass index of 139 ± 14 g/m² in group A was significantly higher than that of 132 ± 12 g/m² in group B ($p < 0.05$), despite the absence of any significant differences in the thickness of the left ventricular wall by M-mode echocardiography.

Blood pressure response during exercise

Peak systolic blood pressure during exercise ranging from 200 to 238 mmHg (219 ± 9.8) in group A was significantly higher than that ranging from 122 to 195 mmHg in group B (176 ± 13.5) ($p < 0.01$). No statistical difference was

Table 1. Clinical Characteristics of Subjects According to Blood Pressure Response During Exercise

	Group A (Exercise hypertension)	Group B (Control)
	No (%)	No (%)
Sex (M/F)	33 (94)/2 (6)	33 (94)/2 (6)
Age (years)	44 ± 8	47 ± 8
Height (cm)	169 ± 7	167 ± 8
Weight (kg)	71 ± 10	68 ± 8
Smoker	15 (43)	12 (34)
Family history of CAD	2 (6)	4 (11)
Fasting glucose (mg/dL)	102 ± 13	99 ± 6
Total cholesterol (mg/dL)	196 ± 34	193 ± 24
Triglyceride (mg/dL)	160 ± 73	130 ± 60
HDL-cholesterol (mg/dL)	46 ± 14	50 ± 14

Data are expressed as the mean ± SD.

CAD, coronary artery disease.

* $p < 0.05$.

noticed in diastolic blood pressure during exercise (90 ± 13.5 in group A vs. 87 ± 13.5 mmHg in group B) or in resting blood pressure. There were no differences in duration of exercise (523 ± 106 in group A vs. 520 ± 76 sec in group B), exercise load in metabolic equivalents (mets) (9.9 ± 1.8 in group A vs. 9.8 ± 1.3 mets in group B) or maximal heart rate (126 ± 28 in group A vs. 135 ± 28 beats/min in group B).

None of the subjects complained angina, palpitation or dizziness and there was no significant ST segment change in EKG during exercise.

Endothelial-dependent vasodilation evaluated by ultrasound

There were no significant differences in the diameter of the brachial artery at rest between the two groups (5.06 ± 0.43 mm in group A vs. 5.04 ± 0.55 mm in group B, $p = \text{NS}$), but a significant decrease in the endothelium-dependent vasodilation was noted in group A compared with group B by reactive hyperemia (3.14 ± 0.61 vs. 6.5 ± 0.76 %, $p < 0.01$). Endothelium-independent vasodilation by sublingual nitroglycerin was lower in group A than in group B, although without statistical significance (8.4 ± 0.7 vs. 10.1 ± 0.78 %,

$p = 0.12$) (Fig. 1). Multiple regression analysis revealed that endothelium-dependent vasodilation was negatively correlated with age ($r = -0.28$, $p < 0.05$) and systolic blood pressure increment during exercise (Δ systolic blood pressure) ($r = -0.36$, $p < 0.01$), but not with weight, fasting serum glucose, lipid profile or cardiac hypertrophy on echocardiography.

Change of $\text{NO}_2^-/\text{NO}_3^-$ and cyclic GMP during exercise

Fourteen randomly selected patients from each group underwent treadmill exercise test up to 60 % of the patient's maximal exercise capacity by the Bruce protocol on the following day after the evaluation of endothelial function by ultrasound, for the measurement of $\text{NO}_2^-/\text{NO}_3^-$ and cyclic GMP. The concentrations of $\text{NO}_2^-/\text{NO}_3^-$ and cyclic GMP were higher during maximal exercise than either those at rest or 30 minutes after exercise (Fig. 2 and 3). Cyclic GMP in group A was significantly lower during exercise than that of group B (8.3 ± 2.5 vs. 10 ± 1.8 pmol/ml, $p < 0.05$) although no significant difference in $\text{NO}_2^-/\text{NO}_3^-$ was noted during exercise between the two groups (55 ± 17 vs. 56 ± 12 $\mu\text{mol/L}$) (Fig. 2 and 3).

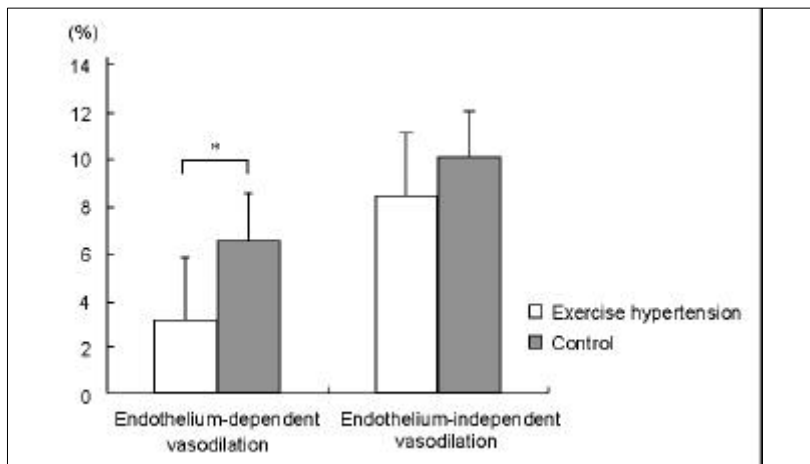


Fig. 1. Endothelium-dependent and -independent vasodilation. Data are expressed as the mean \pm stand error of mean. * $p < 0.05$.

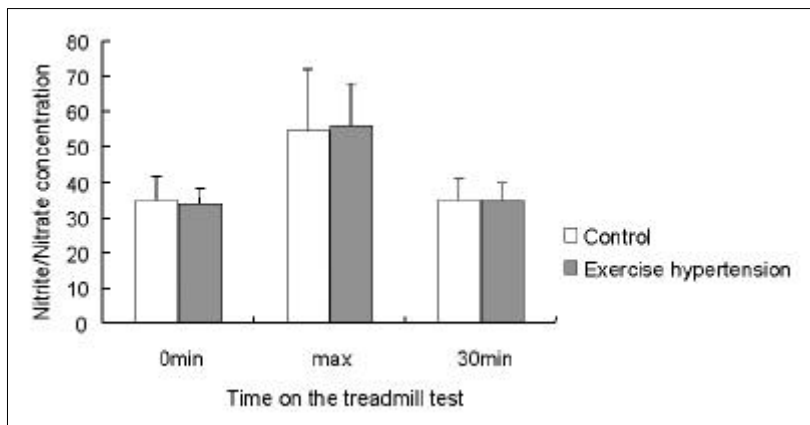


Fig. 2. Differences in the nitrite/nitrate concentration between controls and the exercise hypertension group according to time on the treadmill. Data are expressed as the mean \pm SD. max: maximal treadmill exercise.

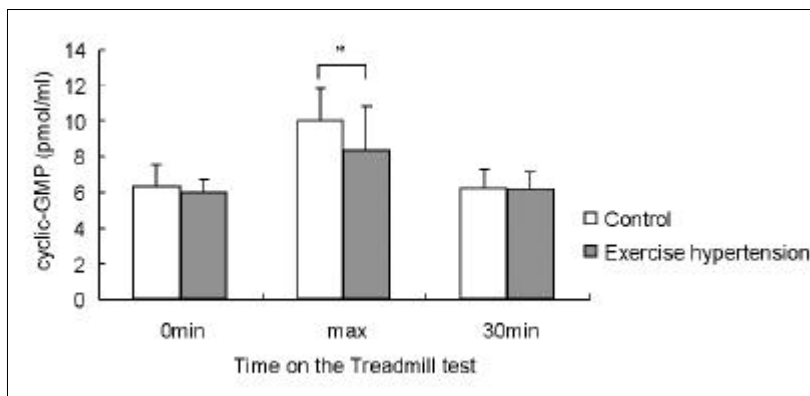


Fig. 3. Differences in the cyclic GMP concentration between controls and the exercise hypertension group according to time on the treadmill. Data are expressed as the mean \pm SD. max: maximal treadmill exercise. * $p < 0.05$.

DISCUSSION

Exercise-induced hypertension is not an uncommon phenomenon in many exercise laboratories. Lauer et al reported that 9% of the normotensive healthy population have exercise-induced hypertension according to the criteria of the Framin-

gham Heart Study, i.e. male ≥ 220 mmHg, female ≥ 190 mmHg.¹²

Mundal, et al.¹⁵ reported that an early rise of systolic blood pressure during exercise adds prognostic information on cardiovascular mortality. Gosse, et al.¹⁶ found that blood pressure measured at maximal exercise is a better pro-

gnostic indicator than resting clinic blood pressure. Recently, Filipovsky, et al.¹⁷ reported, from a survey of 4907 men after 17 years of follow-up, that peak exercise blood pressure is an independent risk factor among otherwise healthy middle-aged men with mildly elevated casual blood pressure.

Levy, et al.¹⁸ reported that left ventricular mass as a harbinger of cardiac morbidity and mortality is more strongly associated with increased systolic blood pressure during exercise than resting blood pressure. Our results also showed that the left ventricular mass and the prevalence of left ventricular hypertrophy on EKG were both increased in the exercise hypertension group. In our study, 68% in the exercise hypertension group, but only 16% in the control group showed left ventricular hypertrophy as defined by a left ventricular index above 134 g/m². This was a similar finding to that reported by Lauer, et al.¹² It appears that frequent loading of high pressure on the left ventricle during exercise may have caused the positive correlation in patients with exercise-induced systolic hypertension and left ventricular mass index.

Wilson, et al.¹⁹ suggested that normotensive individuals at risk for the development of hypertension showed an exaggerated blood pressure response to such physical stimuli as exercise due to poor compliance of peripheral adaptation in proportion to the increment of cardiac output. It has been estimated that the proportion of cardiac output which is distributed in skeletal muscle increases from 15 to 20% of blood volume at rest to approximately 85% during dynamic exercise performed at or near maximal oxygen uptake (VO_{2max}) in humans, which implies the importance of the vascular beds in skeletal muscle, that regulate the cardiovascular system during exercise.²⁰

Traditional explanations for the hyperemia which accompanies exercise have invoked the 'metabolic theory' of vasodilation, whereby contractile activity in the active skeletal muscle gives rise to metabolic by-products which dilate vessels bathed in interstitial fluid.²⁰ However, vasodilation during muscular contraction occurs not only in the microvessels, which are bathed in interstitial fluid and subjected to changes in the level of by-products, but also in larger feed arteries located upstream from the site of metabolic

exchange.²¹

A recent hypothesis is that the locus of blood flow control moves upstream from the microvessels to larger feed arteries as metabolic demand increases. The vascular response to an acute bout of exercise is coordinated by a mechanism which links capillary blood flow to metabolic demand of the skeletal musculature by coupling microvessel dilation with that of upstream arteries. In the presence of such an ascending signal, increases in flow through the microvessels are not limited by perfusion pressure, which is maintained as upstream feed arteries dilate.^{20,22} As a consequence of the increased blood flow velocity through arterial vessels, hydrodynamic drag forces on the endothelium increase and such increases in 'shear' stress stimulate NO release from the endothelium.²⁰

Endothelial cells are responsible for the continuous basal production of NO which serves to counteract neural vasoconstrictor tone and regulate blood flow and pressure.^{5,6} Considering the acute increase of blood flow into the skeletal muscle during exercise, a vasodilatory defect due to endothelial dysfunction might be an important pathophysiologic mechanism of exercise-induced hypertension. In our study, the exercise hypertension group exhibited less endothelium-dependent vasodilation than the control group and its rise of systolic blood pressure was negatively correlated with endothelium-dependent vasodilation.

NO stimulates the release of cyclic GMP, the messenger of NO and Albert, et al.²³ observed a 3-fold increase in serum cyclic GMP level in patients inhaling NO. In our study, there was no difference in the increment of NO during exercise between the two groups. However, there was a defect in endothelium-dependent vasodilation in patients with exercise-induced hypertension, which might be due to an inappropriate increment of cyclic GMP stimulated by NO.

In conclusion, this is the first study, to our knowledge, to demonstrate that an inappropriate endothelium-dependent vasodilation due to inadequate increments of cyclic GMP level among otherwise healthy patients with exercise-induced hypertension. Furthermore, we observed a negative correlation between the rise of systolic blood pressure and endothelium-dependent vasodilation

due to defect in the NO/cyclic GMP pathway. Therefore, rather than being a benign process, exercise-induced hypertension may suggest possible cardiovascular morbidity in the future due to evolving endothelial dysfunction.

REFERENCES

- Singh JP, Larson MG, Manolio TA, O'Donnell CJ, Lauer M, Evans JC, et al. Blood pressure response during treadmill testing as a risk factor for new-onset hypertension. The Framingham Study. *Circulation* 1999;99:1831-6.
- Allison TG, Cordeiro MA, Miller TD, Daida H, Squires RW, Gau GT. Prognostic significance of exercise-induced systemic hypertension in healthy subjects. *Am J Cardiol* 1999;83:371-5.
- Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 2001;104:191-6.
- Chaitman BR. Exercise stress testing. In: Zipes DP, Libby P, Braunwald E, editors. *Heart disease*. 6th ed. Philadelphia, PA: W.B.Saunders Company; 2001. p.129-59.
- Vallance P, Collier J, Moncada S. Effects of endothelium-derived on peripheral arteriolar tone in man. *Lancet* 1989;2:997-1000.
- Stamler JS, Loh E, Roddy MA, Currie KE, Creager MA. Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. *Circulation* 1994;89:2035-40.
- Gilligan DM, Panza JA, Kilcoyne CM, Waclawiw MA, Casino PR, Quyyumi AA. Contribution of endothelium-derived nitric oxide to exercise-induced vasodilation. *Circulation* 1994;90:2853-8.
- Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340:1111-5.
- Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 2000;101:948-54.
- Schachinger V, Britten M, Zeiher A. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000;101:1899-906.
- Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: a critical evaluation. *Clin Chem* 1995;41:892-6.
- Lauer MS, Levy D, Anderson KM, Plehn JF. Is there a relationship between exercise systolic blood pressure response and left ventricular mass? The Framingham Heart Study. *Ann Intern Med* 1992;116:203-10.
- Froelicher VF. *Exercise and the Heart: Clinical concepts*. Chicago Ill: Yearbook Medical Publishers, Inc; 1987. p.102.
- Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 1986;57:450-8.
- Mundal R, Kjeldsen SE, Sandvik L, Erikssen G, Thaulow E, Erikssen J. Exercise blood pressure predicts cardiovascular mortality in middle-aged men. *Hypertension* 1994;24:56-62.
- Gosse P, Durandet P, Roudaut R, Broustet JP, Dalocchio M. Prognostic value of arterial pressure during exertion in hypertensive subjects. *Arch Mal Coeur Vaiss* 1989;82:1339-42.
- Filipovsky J, Ducimetiere P, Safar ME. Prognostic significance of exercise blood pressure and heart rate in middle-aged men. *Hypertension* 1992;20:333-9.
- Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990;322:1561-6.
- Wilson MF, Sung BH, Pincomb GA, Lovullo WR. Exaggerated pressure response to exercise in men at risk for systemic hypertension. *Am J Cardiol* 1990;66:731-6.
- Green DJ, O'Driscoll G, Blanksby BA, Taylor RR. Control of skeletal muscle blood flow during dynamic exercise: contribution of endothelium-derived nitric oxide. *Sports Med* 1996;21:119-46.
- Lash JM. Contribution of arterial feed vessels to skeletal muscle functional hyperaemia. *J Appl Physiol* 1994;76:1512-9.
- Folkow B, Sonnenschein RR, Wright DL. Differential influences of nervous and local humoral factors on large and small precapillary vessels of skeletal muscle. In: Hudlincka O, editor. *Circulation in skeletal muscle*. New York, NY: Pergamon Press; 1966. p.165-9.
- Albert J, Wallen NH, Li N, Frostell C, Hjemdahl P. Neither endogenous nor inhaled nitric oxide influences the function of circulating platelets in healthy volunteers. *Clin Sci (Lond)* 1999;97:345-53.