ORIGINAL ARTICLE

Cardiopulmonary exercise testing and its relation to oxidative stress in patients with hypertension

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An increase in reactive oxygen species has been implicated in the pathologies of hypertension. This study was designed to evaluate antioxidant activity in hypertensive patients and to assess the relationship between oxidative stress and exercise tolerance in hypertensive patients with mild left ventricular diastolic dysfunction (LVDD). A total of 42 patients, aged 51 ± 9 years, with a long history of hypertension and mild LVDD (mitral flow velocities— $E/A < 1$, deceleration time of $E > 220$ ms, and preserved ejection fraction— $EF > 50\%$), and 30 controls without cardiovascular disease, aged 50 ± 7 years, underwent cardiopulmonary exercise testing (CPET). Peak oxygen uptake (peak VO_2), oxygen pulse ($VO_2/\text{heart rate (HR)}$) and ventilatory anaerobic threshold (VAT) were obtained during CPET. Antioxidant activity of superoxide dismutase (SOD) and glutathione peroxidase in the blood was measured before and after exercise. Reduced peak VO_2 (1715 ± 426 vs. 2083 ± 465 ml min⁻¹, $P < 0.001$), VO_2/HR (12.0 ± 2.8 vs. 14.6 ± 3.3 ml per beat, $P < 0.001$) and percentage of peak VO_2 at VAT ($55.5 \pm 15.8\%$ vs. $64.5 \pm 14.7\%$, $P = 0.007$) were observed in hypertensive patients, compared with controls. Antioxidant protection was significantly attenuated in hypertensive patients, compared with controls, before (945 vs. 1006 , $P = 0.012$) and after exercise (954 vs. 1051 , $P < 0.001$). The level of SOD before and after exercise was significantly associated with LVDD in hypertensive patients ($P = 0.012$ and 0.02 , respectively). In addition, the degree of LVDD before exercise (E/A) influenced the degree of exercise capability (peak VO_2) ($P = 0.016$). Asymptomatic hypertensive patients with mild LVDD had reduced cardiopulmonary capacity, accurately identified by CPET. The redox state in hypertensive patients was significantly related to LVDD and exercise tolerance. Attenuated antioxidant protection was associated with long-term hypertension. *Hypertension Research* (2012) 35, 1145–1151; doi:10.1038/hr.2012.115; published online 9 August 2012

Keywords: diastolic dysfunction; exercise capacity; hypertension; superoxide dismutase

INTRODUCTION

Left ventricular diastolic dysfunction (LVDD), which is detected and characterized by Doppler echocardiography, appears early in hypertensive patients, before the onset of abnormal remodeling or left ventricular (LV) hypertrophy, and progressively deteriorates over time.^{1–4} Despite the fact that the initial clinical symptoms predominantly occur during exercise, most studies of hypertensive patients have focused on the parameters of LVDD while at rest.^{2,5–7} Cardiopulmonary exercise testing (CPET) is a recommended technique for the evaluation of the putative mechanism that underlies exercise intolerance in patients with heart failure.^{8,9} In patients with hypertension, impaired LV relaxation and reduced compliance yields inadequate filling at normal diastolic pressures. During exercise, the consequent elevation of left atrial pressure may stimulate receptors associated with augmented ventilation. Elevated

pressures are transmitted to the pulmonary vascular system and stimulate breathing. These cardiac-induced alterations in ventilation may result in reduced exercise tolerance and may cause mild to moderate abnormalities in gas exchange.¹⁰

Exercise enhances the hemodynamic and metabolic response in the body, particularly during acute conditions. An immediate effect of exercise is the multiple-fold increase in oxygen consumption (VO_2). This peak VO_2 boost leads to the production of more free radicals in the plasma and tissues and is followed by a subsequent upregulation of antioxidant defense.^{11–13} Therefore, the enhanced level of antioxidants may contribute to the improvement of oxygen uptake and exercise tolerance. Hypertensive patients in a chronic pro-oxidant state experienced an exacerbation of oxidative stress following exercise, compared with healthy individuals.^{13–15} There are reports of oxidative stress in healthy subjects and diabetics, but there is a

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limited information on the relationship between acute exercise and oxidative stress in hypertensive patients.^{12,13} Although there has been significant research into the effects of regular exercise on the modulation of antioxidant protection, there are limited reports about the role of antioxidants in exercise tolerance.^{13,15} The main source of oxidative stress in hypertension is the superoxide anion, which is normally short-lived due to its rapid reduction to hydrogen peroxide by superoxide dismutase (SOD), the major endogenous vascular enzymatic antioxidant. Glutathione peroxidase (GPx) reduces hydrogen peroxide and lipid peroxides to water and lipid alcohols, respectively, and in turn reduces oxidase glutathione to glutathione disulphide.^{14–16}

The present study was designed to evaluate antioxidant protection and exercise tolerance by CPET in asymptomatic patients with long-standing hypertension and mild LVDD in comparison with non-hypertensive subjects.

METHODS

The study population included 42 patients with long-term hypertension (more than 2 years) who fulfilled Doppler echocardiographic criteria for mild LVDD and preserved LV ejection fraction (LVEF). The criteria for Doppler echocardiography selection included an impaired relaxation ratio of early to late filling (E/A) < 1, a deceleration time of early LV filling > 220 ms, a ratio between mitral and annular early diastolic velocity (E/e) < 12 and a preserved systolic function, that is, LVEF > 50%.^{2,18} Patients were recruited from a larger group of 332 consecutive patients with hypertension who were referred for echocardiography during a 1-year period (2008).

The control group consisted of 30 non-hypertensive subjects without other cardiovascular disease who were matched by age and gender. Exclusion criteria included age > 65 years, moderate to severe pulmonary disease, long-standing diabetes mellitus, rhythm disturbances, electrical pacemakers or implantable cardiac defibrillators, congenital or valvular heart disease, dilated or hypertrophic cardiomyopathy, previous bypass surgery and symptoms of heart failure or renal failure. Patients already involved in any type of training and those who were unable to perform the exercise test were also excluded. Prior to testing, all the subjects gave their written informed consent, and the study was approved by the local Ethics Committee.

Two-dimensional, M-mode and tissue Doppler echocardiography

Doppler echocardiography was performed at rest, prior to exercise testing on the same day, using an Aloka SSD 5000 PHD medical ultrasound system (Aloka Co., Ltd., Tokyo, Japan). The following echo variables were measured or derived: LV dimensions, volumes, LVEF, left atrial dimension and volume, peak transmitral early diastolic (E) velocity, peak transmitral late diastolic velocity (A), E/A ratio, annular myocardial tissue velocity (e) and the E/e ratio. The LV dimension, wall thickness and left atrial dimensions were measured from two-dimensionally derived M-mode echocardiograms. Left ventricle volumes and LVEF were measured using a biplane approach.¹⁷ Peak early and late diastolic transmitral velocities were measured using the pulsed Doppler technique with the sample volume placed at the level of the mitral leaflet tips during diastole in the apical four-chamber view. Myocardial annular tissue velocities were recorded with the pulsed Doppler sample volume positioned within 1 cm of the septal and lateral insertion sites of the anterior and posterior mitral leaflets, respectively.²

Analytical procedures

Activities of the antioxidant enzymes SOD and GPx were assessed as indicators of oxidative stress.^{11,13,16} Antioxidant activity was estimated from blood samples taken before exercise, and 15–20 min after cessation of exercise, which was the suggested time for the objective antioxidant response in normal subjects from previous studies.^{13,18,19} The activity of SOD and GPx ($\text{U g}^{-1} \text{Hb}^{-1}$) was measured by testing the degree of inhibition of the tetrazolium salt oxidation reaction in a 500-nm sample with a commercially available kit (Ransod kit; Randox Laboratories, Crumlin, UK). Study subjects

underwent a minimum of 8 h fasting prior to the blood test to measure glucose and cholesterol levels, and there was no controlled diet in hypertensive patients or controls. Serum biochemical profiles were measured using an autoanalyzer. A mercury sphygmomanometer was used to measure the patient's blood pressure (BP) after 5 min of rest in a quiet environment, with the patient in the sitting position, following the recommendations of the European Society of Hypertension.²⁰

Cardiopulmonary exercise test

Patients underwent exercise testing of metabolic gas exchange by ergospirometry utilizing Jaeger Oxycon Pro (Jaeger, Wuezburg, The Netherlands), with simultaneous heart rate (HR), BP and 12-lead ECG monitoring. The patients performed a continuous symptom-limited maximal test protocol on a bicycle ergometer with an incremental workload of 25 W every 3 min, starting at 25 W.²¹ Patients were encouraged to reach maximal or near maximal effort by monitoring the respiratory exchange ratio (> 1.1). Continuous measurements of respiratory gas exchange (mouthpiece and nose clip) included peak oxygen uptake (peak VO_2), as well as oxygen consumption at the ventilatory anaerobic threshold (VAT), performed by the ventilatory equivalent method using the breath-by-breath system.²¹ Peak oxygen uptake per kg of body weight (peak VO_2/kg), oxygen pulse (peak VO_2/HR), minute ventilation at maximal exercise (peak VE), metabolic equivalents and VE/VCO_2 were also obtained.

Statistical analysis

Data are expressed as the mean $\pm$ s.d. Continuous variables are presented as the mean $\pm$ s.d. Categorical variables are presented as percentages. An independent samples t -test was used to determine the difference between two groups for all clinical, echocardiographic, Doppler and CPET parameters. For correlation of parameters, we used correlation coefficients (r) of Pearson's test in symmetric quantitative variables, whereby a bivariate normal distribution was assumed; Spearman's rho testing was reserved for ordinal scaled categories. The response of SOD and GPx in different groups was evaluated by an analysis of variance. The linear regression model was applied for multivariate analysis. SPSS statistical software (version 15.01, Chicago, IL, USA) was used, and a P -value of < 0.05 (two tailed) was considered significant.

RESULTS

Patient characteristics

The demographic and clinical characteristics of hypertensive patients and controls are presented in Table 1. There were no significant

Table 1 Patients' characteristics

	Hypertension ($n = 42$)	Normotensive ($n = 31$)	P
Age (years)	50.76 $\pm$ 8.95	49.84 $\pm$ 6.94	0.170
Number of smokers	33.3%	40.8%	0.063
Male	38.6%	35.8%	0.210
BMI (kg m^{-2})	26.65 $\pm$ 3.57	26.50 $\pm$ 2.43	0.681
Glucose (mmol l^{-1})	5.80 $\pm$ 1.88	5.69 $\pm$ 0.76	0.424
Cholesterol (mmol l^{-1})	5.69 $\pm$ 1.17	6.01 $\pm$ 1.15	0.090
HDL (mmol l^{-1})	1.11 $\pm$ 0.37	1.16 $\pm$ 0.38	0.540
LDL (mmol l^{-1})	3.7 $\pm$ 0.9	3.9 $\pm$ 1.0	0.434
Triglycerides (mmol l^{-1})	2.91 $\pm$ 0.54	3.1 $\pm$ 0.5	0.144
HR (beats per min)	74.44 $\pm$ 8.88	75.6 $\pm$ 9.9	0.520
SBP (mm Hg)	142.30 $\pm$ 23.32	136.80 $\pm$ 22.50	0.540
DBP (mm Hg)	90.90 $\pm$ 14.62	82.6 $\pm$ 12.63	0.090
VO_2 (ml min^{-1})	376.07 $\pm$ 105.59	402.64 $\pm$ 85.97	0.340
VO_2 per kg ($\text{ml kg}^{-1} \text{min}^{-1}$)	4.89 $\pm$ 1.41	4.85 $\pm$ 1.16	0.560
VE (l min^{-1})	13.19 $\pm$ 3.2	13.06 $\pm$ 3.54	0.519

Abbreviations: BMI, body mass index; DPB, diastolic blood pressure before exercise; HDL, high-density lipoprotein; HR, heart rate; LDL, low-density lipoprotein; SBP, systolic blood pressure before exercise; VE, minute ventilation; VO_2 , peak oxygen consumption.

differences in age, gender, body mass index, glucose and cholesterol level, and number of smokers between study groups. Resting cardiopulmonary parameters were comparable for both groups. Patients were taking the following antihypertensive therapy: angiotensin-converting enzyme inhibitors 29/42 (70%), beta blockers 21/42 (50%), diuretics 19/42 (45%) and calcium antagonists 4/42 (9%). There was no difference in BP between groups at baseline, which indicates that hypertensive medication was keeping the patients' BP controlled.

Doppler echocardiographic findings

Echocardiographic characteristics are presented in Table 2. Hypertensive patients had significantly higher relative wall thickness and slightly reduced LV end-diastolic diameter compared with controls.

Cardiopulmonary exercise capacity

The cardiopulmonary characteristics of subjects from both study groups are presented in Table 3. Maximal HR responses were similar in both groups. BPs were similar at rest (130/90 vs. 125/80 mm Hg, P = not significant), but a significantly higher systolic BP was reached in the hypertensive group at maximal exercise (196 ± 25 vs. 186 ± 21 mm Hg, $P = 0.057$). Hypertensive patients reached signi-

ficantly lower peak VO_2 (1714 ± 426 vs. 2083 ± 465 ml min⁻¹, $P = 0.001$), peak VO_2/HR (12.0 ± 2.8 vs. 14.6 ± 3.3 ml per beat, $P < 0.001$), metabolic equivalent (6.1 ± 1.85 vs. 7.0 ± 1.11 , $P = 0.007$) and percentage of peak VO_2 at VAT ($55.5 \pm 15.8\%$ vs. $64.5 \pm 14.7\%$, $P = 0.007$) compared with controls (Table 3).

Antioxidative protection

Basal level of antioxidant protection according to SOD values before exercise was lower in patients with long-term hypertension, compared with controls (941 ± 120 vs. 1006 ± 163 , $P = 0.096$). The activity of SOD after exercise was attenuated in hypertensive patients (from 941 ± 120 vs. 951 ± 131 , P = not significant), suggesting higher oxidative stress. However, SOD significantly increased in controls from 1006 ± 163 to 1052 ± 170 , $P = 0.026$, therefore, SOD activity in non-hypertensive compared with hypertensive subjects after exercise was significantly different (1052 ± 170 vs. 951 ± 131 , $P < 0.001$), demonstrating a higher antioxidant response in controls. The results obtained by measuring GPx were not so explicit, that is, the level before and after exercise in both groups was not significantly different (hypertensives compared with non-hypertensives before exercise 46.38 ± 12.39 vs. 40.87 ± 12.27 , $P = 0.061$; after exercise 49.00 ± 14.55 vs. 44.80 ± 10.87 , $P = 0.149$). However, differences between levels before and after exercise were significantly higher in non-hypertensive subjects (hypertensives compared with non-hypertensives -3.4 ± 10.49 vs. 3.9 ± 7.60 , $P < 0.001$), suggesting a decreased antioxidant reaction of GPx in hypertensive subjects.

To evaluate the influence of age, smoking status and medication usage on the level of SOD before and after exercise, we used a linear regression model (Tables 4 and 5). Smoking status significantly affected the basal level of SOD only in hypertensive patients and not in controls. Exercise response of SOD activity was not affected by any of these factors. The degree of GPx before and after exercise was not controlled by smoking status, age or medication.

The relationship between exercise capacity, oxidative stress and LV diastolic function in patients with hypertension

There was a significant correlation between exercise capacity as measured by peak VO_2 , and level of SOD, both before ($P = 0.001$) and after exercise ($P = 0.012$) in hypertensive patients, but not in controls (Figure 1).

Table 2 Echocardiographic characteristics of the study patients

	Hypertensive (n = 42)	Normotensive (n = 31)	P
LVEDD (mm)	50.7 $\pm$ 5.0	52.9 $\pm$ 5.4	0.085
LVEDS (mm)	33.8 $\pm$ 6.1	35.6 $\pm$ 6.15	0.144
IVS (mm)	10.8 $\pm$ 1.6	9.4 $\pm$ 1.1	<0.001
PW (mm)	10.5 $\pm$ 1.45	9.4 $\pm$ 1.05	<0.001
EDV/bsa (ml m ⁻²)	64.1 $\pm$ 12.5	63.25 $\pm$ 11.9	0.423
ESV/bsa (ml m ⁻²)	30.9 $\pm$ 7.5	31.7 $\pm$ 7.8	0.814
LVEF (%)	54.3 $\pm$ 7.3	57.2 $\pm$ 6.3	0.061
LA (mm)	38.8 $\pm$ 5.3	37.7 $\pm$ 4.3	0.323
IVRT(s)	108.2 $\pm$ 17.6	103.6 $\pm$ 19.7	0.245

Abbreviations: bsa, body surface area; EDV, end-diastolic volume; ESV, end-systolic volume; IVRT, isovolumic relaxation time; IVS, septum thickness; LA, left atrial dimension; LVEDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; LVEDS, left ventricular end-systolic volume; PW, posterior wall thickness.

Table 3 Data from cardiopulmonary exercise testing

	Hypertensive (n = 42)	Normotensive (n = 31)	P
SBP max (mm Hg)	195.5 $\pm$ 25.4	185.9 $\pm$ 21.0	0.037
DBP max (mm Hg)	98.8 $\pm$ 13.0	96.90 $\pm$ 11.37	0.670
Peak HR (beats per min)	141.31 $\pm$ 16.78	144.16 $\pm$ 15.35	0.088
DP	27513 $\pm$ 4514	26721 $\pm$ 3639	0.569
Time to RER (min)	13.46 $\pm$ 3.37	15.87 $\pm$ 3.94	0.001*
Peak VO_2 (ml min ⁻¹)	1714 $\pm$ 426.14	2082 $\pm$ 465.14	0.001*
Peak VO_2 per kg (ml kg ⁻¹ min ⁻¹)	21.27 $\pm$ 6.49	24.66 $\pm$ 3.89	0.007*
% VO_2 at VAT	55.5 $\pm$ 15.75	64.5 $\pm$ 14.69	0.007*
MET	6.07 $\pm$ 1.85	7.05 $\pm$ 1.11	0.007*
Peak VO_2/HR (ml per beat)	12.05 $\pm$ 2.75	14.57 $\pm$ 3.28	0.001*
VE/ VCO_2	34.43 $\pm$ 3.63	33.23 $\pm$ 4.74	0.508

Abbreviations: DBP max, maximal diastolic blood pressure; DP, double product; HR, peak heart rate; MET, metabolic equivalents; Peak VO_2 , peak oxygen uptake; RER, respiratory exchange ratio; SBP max, maximal systolic blood pressure; VAT, ventilatory anaerobic threshold; VO_2/HR , oxygen pulse; VE/ VCO_2 , ventilation carbon dioxide slope.

* $P < 0.05$.

Table 4 Multivariable model for level of SOD before exercise

	B	P
Non HT		
1		
Constant	1198.185	0.000
Age	-3.302	0.499
Smoking	30.531	0.649
HT		
1		
Constant	852.035	0.006
Age	1.832	0.698
Smoking	-129.414	0.038
ACE inhibitors	-25.599	0.706
Beta blockers	-117.061	0.072
Diuretics	-8.985	0.875

Abbreviations: ACE, angiotensin-converting enzyme; HT, hypertensive patients; non HT, patients without hypertension; SOD, superoxide dismutase.
Dependent variable: SOD before exercise.

Figure 1 Relationship between antioxidant's level and exercise capacity in hypertensive patients.

Figure 2 Relationship between antioxidant's level and LV diastolic function. SOD and velocities of LV diastolic filling.

Table 5 Multivariable model for level of SOD after exercise

	B	P
<i>Non HT</i>		
1		
Constant	884.801	0.012
Age	1.756	0.749
Smoking	-115.395	0.103
<i>HT</i>		
1		
Constant	1035.260	0.000
Age	1.101	0.799
Smoking	104.232	0.089
ACE inhibitors	-51.965	0.421
Beta blockers	-102.104	0.182
Diuretics	82.415	0.347

Abbreviations: ACE, angiotensin-converting enzyme; HT, hypertensive patients; non HT, patients without hypertension; SOD, superoxide dismutase.
Dependent variable: SOD before exercise.

Table 6 Multivariable model for peak VO_2

	B	P
<i>Non HT</i>		
1		
Constant	17.966	0.062
SOD before exercise	-0.002	0.631
LVEDD	0.171	0.225
E/A ratio	-0.099	0.974
<i>HT</i>		
1		
Constant	-10.842	0.461
SOD before exercise	0.030	0.002
LVEDD	0.073	0.719
E/A ratio	-0.323	0.941

Abbreviations: E/A ratio, ratio between early and late LV filling; HT, hypertensive patients; LVEDD, left ventricular end-diastolic dimension; non HT, patients without hypertension; SOD, superoxide dismutase.
Dependent Variable: peak VO_2 .

With regard to the relationship between LVDD and exercise capacity, there was a significant relationship between end-diastolic diameter and volume and peak VO_2 ($r=0.433$, $P<0.001$ and $r=0.513$, $P<0.001$, respectively), as well as between E/A and peak VO_2 ($r=0.265$, $P=0.016$, and peak VO_2/HR , $r=0.344$, $P=0.043$).

Furthermore, a significant positive correlation was observed between antioxidant protection (SOD level) and proper LV diastolic filling, as expressed by the E/A ratio ($r=0.434$, $P=0.005$) (Figure 2), as well as isovolumic relaxation time ($r=-0.402$, $P=0.015$).

From the list of significant correlations, the following parameters of LVDD were entered in a multivariate linear regression model, predicting peak VO_2 in patients with hypertension and in non-hypertensive subjects: E/A ratio, SOD level before exercise and end-diastolic diameter. In the hypertensive group, peak VO_2 was significantly determined by the level of SOD in addition to factors assessing LV filling ($B=0.030$, $P=0.002$) (Table 6).

DISCUSSION

Asymptomatic patients with long-term hypertension and mild LVDD demonstrated lower functional cardiopulmonary capacity than controls, which can be detected early and recognized using CPET. Depressed functional capacity was associated with lower antioxidant protection as measured by SOD level. Interactive relations between oxidative stress, LVDD and exercise tolerance were observed in these patients.

Mechanisms underlying reduced exercise tolerance in hypertension

Recent studies suggest that in patients at rest with isolated LVDD, a combination of systolic and diastolic abnormalities occurs during exercise.^{22,23} In hypertensive patients, mild LVDD at rest was aggravated during exercise, resulting in different modulation of exercise capacity between hypertensive and non-hypertensive subjects.²⁴ Our data suggest that hypertensive patients without

symptoms have significantly reduced functional capacity, for example, lower level of peak VO_2 and VAT, compared with controls. Despite the preserved chronotropic responses, patients with long-term hypertension had reduced maximal aerobic power, largely mediated by an impaired increase in stroke volume and limited peak cardiac output.^{24,25} Additionally, the degree of tissue oxygenation and/or capacity for O_2 utilization may be responsible for different O_2 uptake kinetics in hypertensive and non-hypertensive subjects.²⁶

Hypertension and protection against oxidation

Hypertension is a condition with a permanent shift in redox balance and diminished capacity of the antioxidant defense system.^{14,15} Findings from our study indicate that patients with long-term hypertension have an attenuated SOD level before and after exercise, which is inadequate compared with subjects without hypertension. Low antioxidant bioavailability and decreased content of antioxidant enzymes, including SOD, GPx and catalase, promote cellular oxidative stress and have been implicated in oxidative damage associated with hypertension.^{14,16,27} SOD activity is the first defense against free radical attachment. The O_2^- produced in the mitochondria during ATP generation by oxidative phosphorylation is immediately converted to hydrogen peroxide by mitochondrial manganese SOD (SOD2) and cytosolic copper-zinc SOD (SOD1).¹⁴ Small amounts of SOD are extracellular (SOD3), bound to the extracellular matrix and basal membranes and localized between endothelial and vascular muscle.^{14,27} The SOD1 appears to be expressed in all cells, including erythrocytes, such as the predominant isoform.¹⁵ An important role of cellular SOD in endothelial function is the protection of nitric oxide from oxidative inactivation by superoxide. According to the experimental model, total SOD activity is critically important in protecting the heart from hemodynamic overload.²⁸ In addition, the influences of SOD on phagocyte nicotinamide adenine dinucleotide phosphate oxidase and the nitric oxide family of proteins indicate the central role of SOD in redox-mediated signaling in various cardiovascular cells in hypertensive patients.^{14,16,27,29}

It is difficult to ascertain higher oxidative stress merely due to an increased response in SOD activity; however, SOD is one of the most important indicators of redox balance in hypertensive patients. The study results vary because SOD enzymatic activation occurs through many different mechanisms and its antioxidant activity is a dynamic process. As with our results, in a study directed by Kasperczyk, erythrocyte concentration and SOD activity were significantly higher in normotensive patients than in hypertensive patients.³⁰ Growing evidence suggests that BP modulates measurable oxidant stress-related products, including decreased SOD values.^{14,16,28} On the other hand, a recent study documented higher levels of the biological ROS scavenger SOD in hypertensive subjects compared with normotensive subjects.³¹ Although the reasons have not been fully elucidated, presumably this is a compensatory response to proven high levels of ROS.

Selenium contained in the enzyme GPx catalyzes the removal of hydrogen peroxide through oxidation of reduced glutathione. The dynamic activity of glutathione, including glutathione disulphide, and in turn glutathione oxide, is partially regulated by GPx activity, but other interactions provide many other biochemical pathways.^{14,16} According to our results, the difference in the level of GPx before and after exercise may implicate poorer antioxidant protection in hypertensive patients.

Several factors, such as age, smoking status and the use of some medications, have well-established relationships with oxidative stress

and hypertension.^{32–34} We also found a significant influence of smoking status on the SOD level before exercise in hypertensive patients, but not in controls, suggesting that the impairment of antioxidant defense in hypertensive patients was aggravated by cigarette smoking. However, age in both groups did not affect the results.

There are conflicting results from previous studies about the influence of pharmacological treatment on antioxidant status in hypertensive patients. Most studies have shown a decline in oxidative stress as a result of antihypertensive medications, such as angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists and beta blockers.^{35–37} However, a recent study investigating the role of valsartan on SOD activity demonstrated the profound effect of downregulation of SOD2 in hypertensive patients.³¹ According to our results, there was no significant influence of medication usage on SOD activity in hypertensive patients.

Interactive relations between oxidative stress, LVDD and cardiopulmonary exercise capacity

An important determinant of exercise capacity in healthy volunteers is the downregulation of the pressure/volume curve in early diastole and the consequent increase in LV distension during exercise.^{21,38} Vanoverschelde *et al.*³⁸ demonstrated that the best predictor of exercise capacity in healthy participants was the ratio of early to late transmitral filling velocities. Arruda *et al.*¹⁰ showed that exercise capacity, breathing pattern and gas exchange during exercise are significantly influenced by LV geometry and diastolic function in patients with heart failure and preserved ejection fraction. Altered oxygen uptake and ventilatory efficiency during exercise are related to LV diastolic performance.³⁹

The earliest functional changes in hypertension are the prolongation of isovolumic relaxation, reduced rate of rapid filling and increased amplitude of the A wave, most likely caused by an increase in passive diastolic stiffness. Exercise limitation in patients with LVDD is due to a dynamic impairment of LV active relaxation and compliance.^{5,40} Similarly, in our study, mildly impaired LV filling and depressed LV compliance at rest significantly determined exercise tolerance.

Primary ROS generation in response to acute aerobic exercise can occur via several pathways. A single bout of exercise results in the activation of several distinct systems of free radical generation and these may be separated into both primary sources (for example, electron leakage through the mitochondria during aerobic respiration, prostanoid metabolism, catecholamines and the enzymes xanthine oxidase (perhaps via ischemia-reperfusion conditions) and nicotinamide adenine dinucleotide phosphate oxidase), as well as secondary sources (for example, phagocytic cells, disruption of iron-containing proteins and excessive calcium accumulation). ROS production and the subsequent tissue damage resulting from aerobic exercise are largely due to an increased flux in electron transport, leading to an increased leakage of superoxide radicals.^{13,29}

It appears that several factors influence both the onset of oxidative stress (for example, intensity and duration of exercise, age, smoking, training status and dietary intake) and the detection of such stress *in vivo* (for example, biomarker chosen, tissue sampled and timing of sampling).^{30,31} According to previous studies, there is no best point in time for collecting blood samples after aerobic exercise that applies to all markers.^{13,18} Michailidis *et al.*¹⁸ showed that the optimum post-exercise times for blood collection in untreated individuals are immediately after exercise only for catalase, but not for the other

antioxidants such as SOD and GPx. All these variables may partially explain some of the inconsistency present in the literature.

The potential role of ROS in impairing exercise performance involves altering contractile function and/or accelerating muscle damage and fatigue as a result of the activity of mitochondrial enzymes.^{11–14} The antioxidant defense system, including SOD, may be reduced temporally in response to the exercise pro-oxidant challenge, but in normal conditions, antioxidant response activates during the recovery period.¹³ In long-term disease, such as hypertension, with the progressive production and accumulation of ROS related to a depletion of enzymatic or non-enzymatic antioxidants, antioxidant response is reduced before and after exercise.^{16,27,28} An attenuated antioxidant defense may implicate a lower percentage of maximal aerobic capacity, specifically, peak oxygen consumption in these patients.

There are reports of upregulation in antioxidant defense and alteration of oxidative stress with regular exercise training.^{13,19} Although many studies are concerned with the effects of regular exercise on the modulation of antioxidant protection, there are fewer studies regarding the role of antioxidants in exercise-tolerance variety.^{13,18} According to our results, in patients with long-term hypertension, full exercise capacity was closely related to antioxidant defense, as measured by SOD activity. Furthermore, the level of SOD before exercise was a significant predictor of peak oxygen uptake in hypertensive patients. Attenuated antioxidant defense in hypertensive patients with mild LVDD may be related to changed energetic reserves, oxygen kinetics and uptake.²⁶ Insufficient antioxidant response could be particularly important for understanding the complex and interactive mechanisms underlying the progression from asymptomatic to symptomatic LVDD.^{3,4}

Study limitations

Although we did not perform exercise echocardiography for evaluating actual diastolic dynamics during exercise, we used CPET, which allowed us to assess the contribution of ventilation to functional capacity.

Total antioxidant power or circulating concentrations of antioxidants other than SOD and GPx would provide more insight, but we focused on SOD activity, as it has been shown to have the greatest antioxidant potential in hypertension. Responses in SOD activity may also be due to variations in nitric oxide-mediated O_2^- scavenging, as nitric oxide is the other direct O_2^- scavenger; however, properties of our assay allow us to measure only antioxidant enzymatic activity.

In our clinical setting, we were not able to adequately maintain a controlled diet in study subjects, although the potential effects of diet on hypertension and oxidative stress have previously been reported.

Clinical importance

Detection of functional and antioxidant impairment may be important for the evaluation of asymptomatic hypertensive patients and may control the progression from LVDD to diastolic heart failure. CPET is a reliable, noninvasive and cost-effective technique that ensures accurate assessment of functional impairment in asymptomatic hypertensive patients with isolated LVDD and preserved LVEF. Interrelations between LVDD, cardiopulmonary patterns during exercise and antioxidant protection are applicable to the clinical approach and the medical and exercise prescription. Further studies are required to better understand the role of the redox state in the clinical course of asymptomatic patients with long-term hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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