Original Research

Evaluation of Oxidative Stress Markers and Catecholamine Changes in Patients with Dilated Cardiomyopathy Before and After Cardiopulmonary Exercise Testing

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Introduction: The aim of this study was to evaluate oxidative stress markers (OSM) and catecholamine levels in patients with dilated cardiomyopathy (DCM) before and after cardiopulmonary exercise testing, and to investigate the association between changes in these markers and the New York Heart Association classification (NYHA) and left ventricular ejection fraction (LVEF) in these patients.

Methods: We evaluated 74 patients with DCM and 80 control subjects without DCM. Patients were grouped according to NYHA stages I/II or III/IV. Eligible participants were considered to be those with LVEF values <45%. The OSM analysed included superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPX). The catecholamines analysed included adrenaline, noradrenaline, and dopamine. Vitamin C was also evaluated. All values were obtained before and after cardiopulmonary exercise testing.

Results: There was a significant increase in GR, adrenaline, and noradrenaline after testing in the DCM patients. A significant difference between controls and patients in CAT and evaluated catecholamines was observed after testing. A significant increase in GR, GPX, adrenaline, and noradrenaline for patients in NYHA I/II, and in CAT, GR, adrenaline, noradrenaline, and dopamine for patients in NYHA III/IV, was found between the different times of observation. LVEF before testing showed a significant positive correlation with GPX, and a negative correlation with noradrenaline and adrenaline. After testing a significant negative correlation was found with SOD and GR.

Conclusions: The results of our study demonstrate the complexity of the neurohumoral mechanisms and physiological alterations in the failing heart in DCM patients. Further studies are needed, including other biomarkers and larger samples of patients, in order to improve our understanding of the aetiopathogenesis of DCM development and progression.

Dilated cardiomyopathy (DCM) is a progressive heart muscle disease, characterized by dilatation and systolic dysfunction of the left ventricle, or both ventricles, in the absence of abnormal loading conditions (hypertension, valve disease) or coronary artery disease sufficient to cause global systolic impairment.[1]

It has been stated that oxidative stress plays a significant role in the pathogenesis of DCM.[2] Several studies reported elevated biochemical markers of oxidative stress in adults suffering from DCM in compari-

son to healthy individuals.[3,4] Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant defences that can be either enzymatic or non-enzymatic. In patients with a condition leading to heart failure there are numerous potential sources of ROS: mitochondrial respiratory chain enzymes, NADPH oxidase, xanthine oxidase, neutrophil NADPH oxidase, nitric oxide synthase, etc.[5] It has also been shown that neurohormonal activation (i.e. catecholamines, angiotensin) may enhance oxidative stress in individuals with heart failure.[6] Excessive production of ROS that overcome the anti-oxidative defence capacity might lead to morphological or functional changes in the heart that could be manifested as cardiac dysfunction. Thus, evaluation of the left ventricular ejection fraction (LVEF) might be considered helpful for a better understanding of the influence of oxidative stress on the heart.

Given the above observations, we aimed to evaluate oxidative stress markers and catecholamine changes in patients with DCM before and after cardiopulmonary exercise testing. In addition, we investigated the association between changes in these markers and the New York Heart Association classification (NYHA) and LVEF in DCM patients.

Methods

Study group

This prospective study included 74 patients with DCM who were treated at the Clinical Centre of Serbia (mean age 46.23 ± 17.81 years). We also evaluated 80 randomly selected individuals without DCM, but with similar age distribution (mean 49.73 ± 11.54 years), as a control group. Before their inclusion in the study, eligible participants were informed about the study protocol and consent was obtained. The study was approved by the Institutional Review Board and followed the principles of good clinical practice.

Clinical parameters

The severity of the patients' DCM was assessed in terms of the NYHA classification and LVEF. LVEF (%) was calculated by Simpson's method in 2-dimensional mode, in accordance with the recommendations of the American Society of Echocardiography.[7] Patients in NYHA classes I/II or III/IV, with an LVEF <45%, were eligible for inclusion in the study.[8]

Biochemical parameters

Venous blood samples (5 mL) were obtained in heparinised tubes from each participant on their initial admission to the unit, and 30 and 60 days after the first collection. Samples were immediately centrifuged at 2000 g/10 min/4°C. Supernatants and buffy coats were carefully removed. Separated erythrocytes were washed three times with 0.9% NaCl at 4°C, snap-frozen in liquid nitrogen, and stored at -80°C.

Erythrocytes (0.3 mL) were lysed by adding 3 mL of ice-cold distilled water. Haemoglobin (Hb) concentration was measured by the Drabkin method. Superoxide dismutase (SOD) activity was determined by the adrenaline method.[9] One unit of activity is defined as the amount of enzyme that decreases the rate of adrenalin auto-oxidation at pH 10.2 by 50%. Interference with Hb was eliminated by precipitation prior to the assay using ethanol/chloroform (1:1, v/v) that was followed by centrifugation at 5000 g/5 min/4°C. The catalase (CAT) activity was determined according to the method of Beutler.[10] One unit is defined as the amount of enzyme that reduces 1 μM of H_2O_2 / min. Glutathione peroxidase (GPX) and glutathione reductase (GR) activity was determined as described previously.[11,12] Vitamin C activity was determined according to the method of Okamura.[13] Adrenaline (epinephrine), noradrenaline (norepinephrine), and dopamine were extracted using a cis-diol–specific affinity gel, acylated and then derivatised enzymatically. All the chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Cardiopulmonary exercise testing

Cardiopulmonary exercise testing is a simple and accurate non-invasive method that provides additional useful information for the diagnosis and prognosis in patients with various stage of heart failure. Cardiopulmonary exercise testing was performed using upright graded bicycle exercise (Schiller-Ergosana-CS-200). Heart rate and blood pressure were assessed during defined periods (heart rate during rest and exercise; blood pressure every 2 minutes and at peak exercise).[14]

Statistical analysis

The evaluated parameters were presented as mean values ± standard deviation. For oxidative stress en-

zymes and catecholamines, the statistical difference in values between controls and patients was analyzed using the Mann–Whitney U test. The same test was used to evaluate the statistical significance of differences in selected parameters between different times of observation and between different NYHA groups. The Pearson correlation coefficient (r) was evaluated to determine statistical relationships between the different times of evaluation and between LVEF and the values of other evaluated parameters. Statistical significance was set at $p<0.05$.

Results

The DCM patient population included 38 in NYHA classes I/II and 36 in classes III/IV.

In the control group, a significant increase was observed in CAT ($p<0.01$), GR ($p<0.05$), and GPX ($p<0.05$) enzyme levels, and in all evaluated catecholamines ($p<0.01$) after cardiopulmonary exercise testing (Table 1).

In the patient group, we found a significant increase in GR enzyme levels ($p<0.01$), as well as adrenaline ($p<0.01$) and noradrenaline ($p<0.05$), after cardiopulmonary exercise testing (Table 2). It should be stressed that in this group we observed a decrease in SOD enzyme concentration, even though non-significant, after the cardiopulmonary exercise test (Table 2).

The differences between controls and patients in enzyme concentrations, noradrenaline and dopamine were significant before the cardiopulmonary exercise test (Table 3). After the exercise test, a statistically significant difference was observed in CAT ($p<0.05$) and in the evaluated catecholamines ($p<0.05$) (Table 3).

Before exercise testing, we found that the group of patients in the higher NYHA classes had significantly lower levels of CAT ($p<0.05$) and adrenaline ($p=0.050$), whereas after testing only noradrenaline showed higher levels ($p<0.01$) in patients with higher NYHA classes (Table 4).

There was a significant increase in GR ($p<0.01$), GPX ($p<0.05$), adrenaline ($p<0.05$), and noradrenaline ($p<0.01$) levels in patients in NYHA classes I/II from before to after cardiopulmonary exercise testing (Table 5). Patients in NYHA classes III/IV showed a significant increase in CAT and GR ($p<0.01$ for both), adrenaline and noradrenaline ($p<0.01$ for both), and dopamine ($p<0.05$) from before to after exercise testing) (Table 5).

There was a positive correlation between GPX and LVEF ($r=0.283$, $p=0.040$), and significant negative correlations between noradrenaline and LVEF ($r=-0.294$, $p=0.032$), and between adrenaline and LVEF ($r=-0.345$, $p=0.011$) before cardiopulmonary exercise testing (Table 6). After exercise testing, we observed a significant negative correlation between SOD and LVEF ($r=-0.303$, $p<0.028$), and between GR and LVEF ($r=-0.334$, $p<0.015$) (Table 6).

Discussion

In this study, we demonstrated that there is a significant increase in oxidative stress, particularly in

Table 1. Mean values of evaluated parameters in controls before and after cardiopulmonary exercise testing.

Biochemical parameters	Controls	Mean ± SD	p*
SOD (U/g Hb)	Before	2273.11 ± 799.54	>0.05
	After	2428.92 ± 803.27	
CAT (U/g Hb)	Before	14.79 ± 2.41	<0.01
	After	19.11 ± 4.83	
GR (µmol NADPH/min/g Hb)	Before	4.52 ± 1.05	<0.05
	After	6.31 ± 1.82	
GPX (µmol NADPH/min/g Hb)	Before	12.60 ± 1.91	<0.05
	After	15.79 ± 3.17	
Adrenaline (pg/mL)	Before	28.52 ± 22.0.7	<0.01
	After	146.83 ± 39.77	
Noradrenaline (pg/mL)	Before	317.92 ± 214.63	<0.01
	After	1204.18 ± 878.94	
Dopamine (pg/mL)	Before	26.31 ± 12.18	<0.01
	After	54.38 ± 17.64	
Vitamin C (µM/dm3 plasma)	Before	41.72 ± 35.23	>0.05
	After	42.67 ± 32.91	

*Mann–Whitney U test. SOD – superoxide dismutase; CAT – catalase; GR – glutathione reductase; GPX – glutathione peroxidase.

Table 2. Mean values of evaluated parameters in patients before and after cardiopulmonary exercise testing.

Biochemical parameters	Time	Mean ± SD	p*
SOD (U/g Hb)	Before	3063.05 ± 643.36	>0.05
	After	2946.14 ± 630.38	
CAT (U/g Hb)	Before	12.31 ± 2.42	>0.05
	After	13.29 ± 1.50	
GR (μmol NADPH/min/g Hb)	Before	5.74 ± 1.38	<0.01
	After	6.86 ± 1.38	
GPX (μmol NADPH/min/g Hb)	Before	15.16 ± 2.12	>0.05
	After	15.98 ± 2.40	
Adrenaline (pg/mL)	Before	32.36 ± 21.09	<0.01
	After	114.69 ± 125.57	
Noradrenaline (pg/mL)	Before	501.49 ± 347.25	<0.05
	After	1041.51 ± 768.03	
Dopamine (pg/mL)	Before	71.28 ± 9.94	>0.05
	After	86.87 ± 28.30	
Vitamin C (μM/dm^3 plasma)	Before	35.88 ± 15.18	>0.05
	After	35.80 ± 8.89	

*Mann–Whitney U test. Abbreviations as in Table 1.

Table 3. Statistical significance of differences in evaluated parameters between patients and controls, before and after cardiopulmonary exercise testing.

Biochemical parameters	p-value*	
	Before	After
SOD	<0.05	>0.05
CAT	<0.05	<0.05
GR	<0.05	>0.05
GPX	<0.01	>0.05
Adrenaline	>0.05	<0.05
Noradrenaline	<0.01	<0.05
Dopamine	<0.05	<0.05
Vitamin C	>0.05	>0.05

*Mann–Whitney U test. Abbreviations as in Table 1.

healthy controls, after cardiopulmonary exercise testing. Our findings are consistent to a certain degree with those of previous studies that found a correlation between exercise testing and an increase in oxidative stress in healthy individuals.[15,16] As for oxidative stress markers, in our study there was a significant increase in catecholamine values evaluated after exercise testing in healthy controls. Such findings are also consistent with previous reports.[17] However, it should be underlined that there are likely to be individual variations in the response of oxidative stress and catecholamine levels to exercise, particularly associated with age, intensity of training, and adaptation levels.[15,16,17]

In our study, we demonstrated that patients with DCM showed a significant increase in the levels of several catecholamines between the different times of evaluation (beginning and end of cardiopulmonary exercise testing), whereas of all the oxidative stress enzymes evaluated, only GR showed such a significant change. A significant increase in levels of noradrenaline and adrenaline after exercise in patients with congestive heart failure was observed in the study of Madsen et al.[18] It was also suggested that plasma noradrenaline during the resting period might be a significant prognostic factor for survival.[18]

It is important to note that noradrenaline and dopamine levels were significantly higher in controls than in DCM patients before and after exercise testing, while a significant increase in adrenaline levels after exercise testing was observed only in the patient group. These findings imply that DCM may influence the production of catecholamines to some degree. Since patients with DCM might frequently be in various states of hypoxia, previous studies demonstrated that such a state could trigger an increase in adrenaline secretion.[17] Therefore, hypoxia might be one of the possible factors causing an increase of adrenaline levels during exercise testing in patients with DCM. However, further studies are needed to confirm this hypothesis.

Our findings suggest that, during cardiopulmonary exercise testing in patients with DCM versus controls, changes in catecholamine levels are more significant than changes in the levels of oxidative

Table 4. Differences in evaluated parameters in relation to NYHA class before and after cardiopulmonary exercise testing.

Biochemical parameters	Time	NYHA	Mean ± SD	p*
SOD (U/g Hb)	Before	NYHA I/II	3242.64 ± 336.45	>0.05
		NYHA III/IV	2965.71 ± 788.90	
	After	NYHA I/II	2969.45 ± 675.99	>0.05
		NYHA III/IV	3063.14 ± 464.96	
CAT (U/g Hb)	Before	NYHA I/II	13.34 ± 2.02	<0.05
		NYHA III/IV	11.66 ± 2.48	
	After	NYHA I/II	13.77 ± 1.31	>0.05
		NYHA III/IV	13.16 ± 1.34	
GR (μmol NADPH/min/g Hb)	Before	NYHA I/II	6.22 ± 1.73	>0.05
		NYHA III/IV	5.88 ± 1.33	
	After	NYHA I/II	6.87 ± 1.01	>0.05
		NYHA III/IV	7.55 ± 1.74	
GPX (μmol NADPH/min/g Hb)	Before	NYHA I/II	14.77 ± 1.67	>0.05
		NYHA III/IV	15.68 ± 2.51	
	After	NYHA I/II	16.18 ± 2.38	>0.05
		NYHA III/IV	16.33 ± 2.50	
Adrenaline (pg/mL)	Before	NYHA I/II	43.73 ± 33.32	≈0.05
		NYHA III/IV	28.36 ± 6.51	
	After	NYHA I/II	114.14 ± 122.28	>0.05
		NYHA III/IV	125.91 ± 153.19	
Noradrenaline (pg/mL)	Before	NYHA I/II	370.74 ± 252.09	>0.05
		NYHA III/IV	578.69 ± 403.42	
	After	NYHA I/II	668.04 ± 601.62	<0.01
		NYHA III/IV	1340.26 ± 876.29	
Dopamine (pg/mL)	Before	NYHA I/II	75.48 ± 15.15	>0.05
		NYHA III/IV	69.68 ± 4.20	
	After	NYHA I/II	83.80 ± 26.02	>0.05
		NYHA III/IV	87.07 ± 29.66	
Vitamin C (μM/dm^3 plasma)	Before	NYHA I/II	42.37 ± 13.98	>0.05
		NYHA III/IV	36.89 ± 16.34	
	After	NYHA I/II	37.78 ± 7.72	>0.05
		NYHA III/IV	32.83 ± 9.66	

*Mann–Whitney U test. NYHA - New York Heart Association classification. Other abbreviations as in Table 1.

stress enzymes. It appears that patients with DCM are under higher oxidative stress compared to controls. When the values of oxidative stress markers in DCM patients were compared with those of controls before and after exercise testing, we found significant changes after testing only for CAT. This observation is consistent with previous reports that pointed out the role of numerous factors that might influence such an increase in both DCM patients and patients with heart failure.[19,20]

The significantly higher GPX levels in the DCM patients versus controls before testing might suggest an up-regulation in oxidative stress reactions towards a more protective state, since GPX is a key antioxidant that catalyzes H_2O_2 and hydroperoxide reduction, and might have a stronger protective effect than SOD, since in the course of oxidative reactions SOD might cause an increase in H_2O_2.[21] The importance of GPX is that it could be considered to be of primary importance in heart protection, since it is found in larger amounts in the myocardium.[21,22]

Among the oxidative stress enzymes evaluated in our study, significant differences before cardiopulmonary exercise testing between patients with disease of different clinical severity, according to the NYHA classification, were found only for CAT. The absence of a significant increase in GPX levels in patients with severe DCM, according to NYHA class, could sug-

Table 5. Statistical significance of changes in evaluated parameters before versus after cardiopulmonary exercise testing in relation to NYHA class.

Biochemical parameters	p-value*	
	NYHA I/II	NYHA III/IV
SOD	>0.05	>0.05
CAT	>0.05	<0.01
GR	<0.01	<0.01
GPX	<0.05	>0.05
Adrenaline	<0.05	<0.01
Noradrenaline	<0.01	<0.01
Dopamine	>0.05	<0.05
Vitamin C	>0.05	>0.05

*Mann–Whitney U test. Abbreviations as in Tables 1 & 4.

Table 6. Correlation between evaluated parameters and left ventricular ejection fraction before and after cardiopulmonary exercise testing.

Biochemical parameters	Before		After	
	r	p	r	p
SOD	0.021	0.883	-0.303	0.028
CAT	-0.162	0.246	0.073	0.602
GR	-0.225	0.105	-0.334	0.015
GPX	0.283	0.040	-0.092	0.514
Adrenaline	-0.345	0.011	-0.083	0.556
Noradrenaline	-0.294	0.032	-0.124	0.375
Dopamine	-0.224	0.107	0.155	0.267
Vitamin C	0.157	0.261	0.136	0.331

Abbreviations as in Table 1.

gest that other factors might play some role in the aetiopathogenesis of DCM evolution and progression.

Among the catecholamines, adrenaline values were significantly higher in the group with less severe disease (NYHA I/II) before testing, while such a difference was not observed after the test. In contrast to this observation, even though we found a non-significant difference in noradrenaline levels between NYHA I/II and NYHA III/IV patients before exercise testing, after the cardiopulmonary exercise test the NYHA III/IV group had significantly higher values. Our findings from before the cardiopulmonary exercise test are consistent with previous reports indicating that catecholamines in patients with DCM showed no significant differences in relation to NYHA class.[23] The significant differences in adrenaline levels between the groups of DCM patients with NYHA I/II and NYHA III/IV in our study may have been due to variations in the sympathetic response among the individuals studied. Another possibility is that the difference was related to the patients' treatment regimen, since it has been previously reported that beta adrenergic blockade (selective and non-selective) can change catecholamine levels.[24] This might be explained by the fact that beta adrenergic receptors can modulate efferent sympathetic nerve activity, along with the release of norepinephrine from adrenergic nerve terminals.[24] Similar reasoning could be applied to the significant differences in noradrenaline values between NYHA I/II and NYHA III/IV patients after cardiopulmonary exercise.

It has previously been suggested that numerous biomarkers, including those of oxidative stress, might be associated with cardiac dysfunction and the degree of congestive heart failure.[6,25-27] We have demonstrated that, to a certain degree, various oxidative stress markers are significantly affected by the degree of DCM severity according to NYHA class. Similar findings were observed for catecholamines, where we observed significant changes only for dopamine in the group with more severe DCM (NYHA III/IV). The significant increase in the evaluated catecholamines, including dopamine, after cardiopulmonary exercise testing in DCM patients with more severe disease, compared to those with a lower degree of severity (significant changes were sought for adrenaline and noradrenaline), might be explained by the fact that in those with NYHA III/IV, sympathetic activity is increased more, which in turn raises catecholamine levels and may worsen myocardial injury leading to the progression of DCM.

Previous studies in animal models demonstrated that catecholamines could increase the level of ROS.[28] When cardiac dysfunction is present in patients with DCM, who thus have a reduced LVEF, there is a compensatory increase in sympathetic nervous system activity,[29] which in turn could alter catecholamine levels. Our results showing that catecholamine levels significantly correlate with LVEF, with a lower LVEF being associated with higher values of catecholamines (noradrenaline and adrenaline), are in concordance with previous reports. However, after cardiopulmonary exercise testing we found only non-significant correlations between the assessed catecholamine levels and LVEF, suggesting the possible assumption that other compensatory mechanisms might be activated during the period of exercise activity, preventing a significant increase or decrease in assessed catecholamine levels in relation to the severity of LVEF dysfunction. It should be noted that, after

cardiopulmonary exercise, SOD and GR enzyme levels were significantly correlated with the severity of LV dysfunction (higher SOD and GR associated with lower LVEF). Our findings might suggest that a significant correlation between the evaluated oxidative stress enzyme levels and LVEF could be due to the fact that, over the increased period of physical activity, more ROS are produced and that the compensatory mechanisms have less capacity to overcome such a state, with a consequent rise in the levels of oxidative stress enzymes. The possible increased production of ROS might also be associated with increased catecholamine levels (before exercise testing in our study) and thus represent physiological disequilibrium in DCM patients.

Given the above observations, it is clear from the results of our study how complex are the neurohumoral mechanisms and physiological alterations in the failing heart in patients who suffer from DCM. The modifications in the physiological equilibrium of numerous systems in DCM patients might be the result of achieving maximal heart morphology preservation through changes in the reaction of compensatory mechanisms. Therefore, further studies are needed, including other biomarkers and larger samples of patients, in order to improve our understanding of the aetiopathogenesis of DCM development and progression.

References

1. Elliott P, Andersson B, Arbustini E, et al. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2008; 29: 270-276.
2. Bajcetic M, Kokic Nikolic A, Djukic M, et al. Effects of carvedilol on left ventricular function and oxidative stress in infants and children with idiopathic dilated cardiomyopathy: a 12-month, two-center, open-label study. Clin Ther. 2008; 30: 702-714.
3. Kono Y, Nakamura K, Kimura H, et al. Elevated levels of oxidative DNA damage in serum and myocardium of patients with heart failure. Circ J. 2006; 70: 1001-1005.
4. Gopal DM, Sam F. New and emerging biomarkers in left ventricular systolic dysfunction—insight into dilated cardiomyopathy. J Cardiovasc Transl Res. 2013; 6: 516-527.
5. Wattanapitayakul SK, Bauer JA. Oxidative pathways in cardiovascular disease: roles, mechanisms, and therapeutic implications. Pharmacol Ther. 2001; 89: 187-206.
6. Korantzopoulos P, Galaris D, Papaioannides D, Siogas K. The possible role of oxidative stress in heart failure and the potential of antioxidant intervention. Med Sci Monit. 2003; 9: RA120-125.
7. Schiller NB, Shah PM, Crawford M, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr. 1989; 2: 358-367.
8. Habash-Bseiso DE, Rokey R, Berger CJ, Weier AW, Chyou PH. Accuracy of noninvasive ejection fraction measurement in a large community-based clinic. Clin Med Res. 2005; 3: 75-82.
9. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247: 3170-3175.
10. Beutler E. Red Cell Metabolism. A Manual of Biochemical Methods. New York: Grune and Stratton, Inc, 1982.
11. Paglia DE, Valentine NW. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967; 70: 158-169.
12. Glatzle D, Vuilleumier JP, Weber F, Decker K. Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in humans. Experientia. 1974; 30: 665-667.
13. Okamura M. An improved method for determination of L-ascorbic acid and L-dehydroascorbic acid in blood plasma. Clin Chim Acta. 1980; 103: 259-268.
14. Guazzi M, Labate V, Cahalin LP, Arena R. Cardiopulmonary exercise testing reflects similar pathophysiology and disease severity in heart failure patients with reduced and preserved ejection fraction. Eur J Prev Cardiol. 2014; 21: 847-854.
15. Bouzid MA, Hammouda O, Matran R, Robin S, Fabre C. Changes in oxidative stress markers and biological markers of muscle injury with aging at rest and in response to an exhaustive exercise. PLoS One. 2014; 9: e90420.
16. Kyparos A, Vrabas IS, Nikolaidis MG, Riganas CS, Kouretas D. Increased oxidative stress blood markers in well-trained rowers following two thousand-meter rowing ergometer race. J Strength Cond Res. 2009; 23: 1418-1426.
17. Zouhal H, Jacob C, Delamarche P, Gratas-Delamarche A. Catecholamines and the effects of exercise, training and gender. Sports Med. 2008; 38: 401-423.
18. Madsen BK, Keller N, Christiansen E, Christensen NJ. Prognostic value of plasma catecholamines, plasma renin activity, and plasma atrial natriuretic peptide at rest and during exercise in congestive heart failure: comparison with clinical evaluation, ejection fraction, and exercise capacity. J Card Fail. 1995; 1: 207-216.
19. Wojciechowska C, Romuk E, Tomasik A, et al. Oxidative stress markers and C-reactive protein are related to severity of heart failure in patients with dilated cardiomyopathy. Mediators Inflamm 2014; 2014: 147040.
20. White M, Ducharme A, Ibrahim R, et al. Increased systemic inflammation and oxidative stress in patients with worsening congestive heart failure: improvement after short-term inotropic support. Clin Sci (Lond). 2006; 110: 483-489.
21. Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. Am J Physiol Heart Circ Physiol. 2011; 301: H2181-2190.
22. Le CT, Hollaar L, van der Valk EJ, van der Laarse A. Buthionine sulfoximine reduces the protective capacity of myocytes to withstand peroxide-derived free radical attack. J Mol Cell Cardiol. 1993; 25: 519-528.
23. Agarwal AK, Venugopalan P, Woodhouse C, de Bono D. Catecholamine levels in heart failure due to dilated cardiomyopathy and their relationship to the severity of heart fail-

ure. Eur J Heart Fail. 2000; 2: 261-263.
24. Newton GE, Parker JD. Acute effects of beta 1-selective and nonselective beta-adrenergic receptor blockade on cardiac sympathetic activity in congestive heart failure. Circulation. 1996; 94: 353-358.
25. Belch JJ, Bridges AB, Scott N, Chopra M. Oxygen free radicals and congestive heart failure. Br Heart J. 1991; 65: 245-248.
26. Kakouros NS, Kakouros SN. Clinical assessment in acute heart failure. Hellenic J Cardiol. 2015; 56: 285-301.
27. Perpinia A, Tsounis D, Karavidas A, et al. Diagnostic value of soluble adhesion molecule kinetics in patients with suspected myocardial ischemia undergoing dobutamine stress echocardiography. Hellenic J Cardiol. 2014; 55: 322-327.
28. Amin JK, Xiao L, Pimental DR, et al. Reactive oxygen species mediate alpha-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes. J Mol Cell Cardiol. 2001; 33: 131-139.
29. Rochette L, Tatou E, Maupoil V, et al. Atrial and vascular oxidative stress in patients with heart failure. Cell Physiol Biochem. 2011; 27: 497-502.