Relation Between Oxidative Stress, Catecholamines, and Impaired Chronotropic Response to Exercise in Patients With Chronic Heart Failure Secondary to Ischemic or Idiopathic Dilated Cardiomyopathy

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Oxidative stress has been implicated in the pathogenesis of chronic heart failure (HF). Different studies have shown that reactive oxygen species are produced in the failing myocardium, causing injury in cardiac myocytes.[1–3] Different factors classically associated with cardiomyocyte dysfunction and death in chronic HF, such as increased plasma catecholamine levels, are well-known stimuli for the generation of reactive oxygen species.[4] In patients with advanced chronic HF at rest, the circulating norepinephrine concentrations are much higher, generally 2 to 3 times the level found in normal subjects.[5,6] During comparable levels of exercise, much greater elevations in circulating norepinephrine occur in patients with chronic HF than in normal subjects. However, despite the increase in norepinephrine with exercise, patients with chronic HF had an attenuated heart rate response to exercise; this finding has been attributed to postsynaptic desensitization of the β-adrenergic receptor pathway.[7] A relation between cardiac exercise capacity and oxidative stress determined by malondialdehyde (MDA) plasma levels, a marker of lipid peroxidation, has been proposed.[8] Experimental data also suggest that hydrogen peroxide may attenuate the β-adrenoceptor–linked signal transduction in the heart by changing the functions of Gs proteins and the catalytic subunit of the adenylyl cyclase.[9] In the present study we investigated the association between MDA plasma levels, catecholamines at peak exercise, and impaired heart rate response to exercise in patients with chronic HF.

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We enrolled 27 patients with chronic HF secondary to coronary heart disease (n = 15) or idiopathic dilated cardiomyopathy (n = 12). They fulfilled the following criteria: (1) chronic stable HF in New York Heart Association functional classes II to IV; (2) ability to complete a symptom-limited treadmill exercise test; (3) evidence of left ventricular (LV) dilation and LV ejection fraction <40% as determined by radionuclide-gated pool scan; and (4) treatment with diuretics, digitalis, and vasodilators. We excluded patients with (1) coronary artery bypass surgery, angioplasty, or myocardial infarction in the last 6 months; (2) chronic angina; (3) uncontrolled hypertension (systolic blood pressure >160 mm Hg or diastolic blood pressure >90 mm Hg); (4) hypertensive myocardiopathy; (5) change in maintenance therapy or use of β blockers in the last 2 months; (6) implanted pacemaker; (7) significant valvular disease; and (8) presence of other conditions that affect determination of oxidative stress status, such as renal insufficiency (plasma creatinine >2 mg/dl), autoimmune diseases, neoplasia, advanced liver or pulmonary disease, and acute or chronic inflammation.

All patients signed an informed consent approved by our institutional review board and ethics committee. For clinical assessment we used New York Heart Association functional class and the Mahler et al[10] clinical score (range 0 to 12 points), which evaluates the severity of dyspnea. The score depends on ratings for 3 different categories: functional impairment, magnitude of task, and magnitude of effort. Dyspnea is rated in 5 degrees from 0 (severe) to 4 (unimpaired) for each category. The ratings for each of the 3 categories are added to form the score.

LV end-diastolic and LV end-systolic diameters were determined by Doppler 2-dimensional echocardiography, and LV ejection fraction was determined by radionuclide ventriculography. Each patient performed a 6-minute corridor walk test and a maximal exercise test with gas exchange.

Plasma norepinephrine and epinephrine specimens were collected from an indwelling venous line after patients had been in the supine position in a quiet room for 30 minutes. Measurements were repeated at maximal exercise. Determination of catecholamines was performed by high-performance liquid chromatography using a commercial kit (Chromsystems Instruments & Chemicals GmbH, München, Germany). The inter- and intra-assay coefficients were 6% and 5%, respectively. The ratio of the increment in heart rate divided by the increment in norepinephrine from

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TABLE 1 Clinical Characteristics of Patients With Heart Failure (HF) (n = 27)

Characteristics	
Age (mean ± SEM) (yrs)	60 ± 3
Men	24 (89%)
Etiology	
Ischemic	15 (56%)
Idiopathic	12 (44%)
History of	
Myocardial infarction	9 (33%)
Coronary bypass surgery	5 (19%)
Percutaneous coronary angioplasty	1 (4%)
Diabetes mellitus*	3 (11%)
Systemic hypertension	12 (44%)
Hypercholesterolemia*	7 (26%)
Current smoking	7 (26%)
Chronic therapy	
Angiotensin-converting enzyme inhibitors†	20 (74%)
Losartan†	2 (7%)
Hidralazine + isosorbide	4 (15%)
Furosemide	23 (85%)
Spironolactone	16 (59%)
Digoxin	18 (67%)
LV ejection fraction (mean ± SEM)	25 ± 1.6%
LV end-diastolic diameter (mean ± SEM) (mm)	70 ± 2.2
LV end-systolic diameter (mean ± SEM) (mm)	58 ± 2.3

*Hypercholesterolemia and diabetes were defined by a total cholesterol level ≥240 mg/dl and by a total glucose level ≥100 mg/dl.
†Mean doses of angiotensin-converting enzyme inhibitors (in enalapril equivalents) and losartan were 19 ± 3 and 56 ± 16 mg, respectively.

TABLE 2 Functional Evaluations of Patients With Heart Failure (HF)

Functional Evaluation	
NYHA functional class	
II	16 (59%)
III	10 (37%)
IV	1 (4%)
Mahler score (mean [median])	7 [6]
6-min walk test × (m)	489 ± 25
Cardiopulmonary exercise test	
Peak VO_2 (ml/kg/min)	19.1 ± 1.7
Anaerobic threshold (ml/kg/min)	15.2 ± 1.3
Duration (min)	16.1 ± 1.9

Values are expressed as mean ± SEM unless otherwise noted.
NYHA = New York Heart Association; VO_2 = oxygen consumption rate.

rest to peak exercise was used as an index of chronotropic responsiveness.[7]

Determinations of oxidative stress and antioxidant enzyme activities were performed before exercise. MDA was determined by examining the content of thiobarbituric acid-reactive substances as previously described.[11] A standard commercially available MDA (Merck, Darmstad, Germany) was used. Superoxide dismutase was extracted from the lysates according to the method of Mc Cord and Fridovich,[12] and its activity was assayed as described by Misra and Fridovich.[13] Catalase was determined by the method described by Beers and Sizer.[14] Glutathione peroxidase was determined as described Paglia and Valentine.[15] All enzymatic activities were expressed as units per gram of hemoglobin.

Oxidative stress parameters were compared with a control group of 15 healthy, age- and gender-matched volunteers. They were all asymptomatic, with a normal medical history and a normal physical examination. Control subjects were excluded if they had any known coronary risk factor, if they were taking any medications, vitamin supplements, antioxidants, or if they drank alcohol on a regular basis.

Results are presented as mean ± SEM. We used the Student's *t* test for paired samples for comparing resting with peak exercise catecholamine levels. Linear regression analysis was performed to evaluate the correlations between oxidative stress parameters, catecholamine levels, and the index of chronotropic responsiveness. A p value ≤0.05 was considered significant.

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The group consisted of 27 patients with chronic HF (24 men and 3 women, mean age 60 ± 3 years [range 47 to 71]). Twenty-six patients had New York Heart Association class II or III chronic HF. Fifteen patients had ischemic dilated cardiomyopathy (6 of whom underwent previous myocardial revascularization surgery). Twelve patients had idiopathic dilated cardiomyopathy (Table 1). Twenty patients were in sinus rhythm and 7 were in atrial fibrillation. In addition, 9 patients had left bundle branch block and 2 had right bundle branch block. Nine patients had evidence of previous myocardial infarction by electrocardiographic criteria. LV function parameters are listed in Table 1.

The mean Mahler score was 7 points (median 6), and the 6-minute walk test ranged from 275 to 660 m (mean 489 ± 25) (Table 2). Cardiopulmonary exercise test data are listed in Table 2.

The 6-minute walk distance and the peak oxygen consumption rates were negatively correlated with norepinephrine determinations at rest ($r = -0.52$, $p = 0.04$ and $r = -0.49$, $p = 0.03$, respectively). The oxidative stress parameters are listed in Table 3. Patients with chronic HF had a significant elevation in MDA levels compared with controls (2.6 ± 0.2 vs 0.9 ± 0.1 μM, $p < 0.001$, respectively). Catalase activities (patients vs controls: 121 ± 16 vs 193 ± 8 U/g of hemoglobin, $p = 0.003$) decreased, whereas glutathione peroxidase activities increased (patients vs controls: 57 ± 4 vs 33 ± 1 U/g of hemoglobin, $p < 0.001$). Superoxide dismutase activities tended to decrease in patients, but the difference was not significant (patients vs controls: 1.0 ± 0.1 vs 1.3 ± 0.1 U/g of hemoglobin, $p = 0.07$).

The mean baseline plasma levels of epinephrine and norepinephrine were 23 ± 2 and 531 ± 73 pg/ml and increased significantly with exercise (92 ± 19 and 2,752 ± 282 pg/ml, $p = 0.005$ and $p < 0.001$, respectively) (Table 4). There was no relation between MDA levels at rest and norepinephrine at rest ($r = -0.20$, $p = 0.385$) or epinephrine ($r = 0.215$, $p = 0.337$). There was a significant correlation between MDA plasma levels and peak exercise determinations of norepinephrine ($r = 0.57$, $p = 0.01$) but not with epinephrine ($r = 0.35$, $p = 0.17$). There was no correlation be-

TABLE 3 Oxidative Stress Parameters in Patients With Heart Failure (HF) and in Control Subjects

	Patients		Controls		
	Value ± SEM	Range	Value ± SEM	Range	p Value
MDA (μmol/L)	2.6 ± 0.2	0.5–5.9	0.9 ± 0.1	0.3–1.7	<0.001
Catalase (U/g of Hb)	121 ± 16	32–353	193 ± 8	145–242	0.003
Superoxide dismutase (U/g of Hb)	1.0 ± 0.1	0.3–2.5	1.3 ± 0.1	0.7–2.2	0.07
Glutathione peroxidase (U/g of Hb)	57 ± 4	19–112	33 ± 1	24–41	<0.001

Hb = hemoglobin.

TABLE 4 Catecholamine Plasma Levels in Patients With Heart Failure (HF) Before and at Peak Exercise

	Before Exercise		Peak Exercise		
	Mean ± SEM	Range	Mean ± SEM	Range	p Value
Epinephrine (pg/ml)	23 ± 2	20–44	92 ± 19	20–257	0.005
Norepinephrine (pg/ml)	531 ± 73	222–1,431	2752 ± 282	1,189–5,487	<0.001

tween MDA determinations and LV ejection fraction, exercise parameters, or dosages of angiotensin-converting enzyme inhibitors (data not shown).

There was a significant negative correlation between the index of chronotropic responsiveness and MDA plasma levels ($r = -0.58$, $p = 0.015$). No significant correlations were found between catecholamines and antioxidant enzyme activities or between antioxidant enzyme activities and the index of chronotropic responsiveness (data not shown).

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In the present study, we found that patients with stable chronic HF had increased oxidative stress, as determined by higher plasma MDA. Higher levels of MDA correlated with the catecholaminergic response to exercise and with a decreased index of chronotropic responsiveness.

The link between oxidative stress and adrenergic stimulation is based on experimental animal models and clinical experiences. Kharper et al,[16] working on a rat model of myocardial infarction, demonstrated a protective effect of propanolol after reperfusion. This effect was associated with a reduction in MDA levels and higher activities in catalase and glutathione peroxidase. Kukin et al[17] observed a reduction in thiobarbituric acid-reactive substances after 6 months of therapy with the β blockers metoprolol and carvedilol in 67 patients with symptomatic stable chronic HF. Nakamura et al[4] detected elevated myocardial oxidative stress in myocardium of patients with chronic HF. Administration of carvedilol resulted in a decrease in the oxidative stress level together with amelioration of cardiac function.

There is ample evidence that sympathetic nervous system activity at rest is increased in patients with chronic HF.[7] Moreover, at comparable degrees of peak oxygen consumption, plasma norepinephrine was similar or greater in patients with chronic HF.[18]

In our patients, the magnitude of norepinephrine elevation at peak exercise was correlated with the degree of oxidative stress, as determined by MDA plasma levels at rest. However, we did not find a correlation between MDA and LV ejection fraction or exercise intolerance. The absence of a correlation between MDA plasma levels and LV ejection fraction was also described by Díaz-Velez et al.[19] However, a significant inverse correlation between exercise-induced changes in MDA and peak oxygen consumption rate was described.[8]

Patients with chronic HF have an attenuated heart rate response to peak exercise. This finding has been attributed to desensitization of the β-adrenergic pathway in the sinoatrial node. During exercise, at any given increase in norepinephrine levels, heart rate was found to be lower in patients with chronic HF. Because increases in heart rate are mediated primarily by the action of the sympathetic nervous system on β-adrenergic receptors,[7] these data suggest that the sinoatrial node may be less sensitive to β-adrenergic stimulation in patients with chronic HF. The nature of this attenuated response to exercise could not be elucidated by this study, but some experimental data have shown a depression in the isoproterenol-stimulated adenylyl cyclase activity in heart membranes treated with high concentrations of hydrogen peroxide.[9]

The main limitations of the present study are the small number of patients studied and the absence of a control group. We also included some patients with atrial fibrillation; in these patients the mechanism of rate response to exercise depends on catecholamines, mainly by the vagal tone. Despite this limitation, the correlations were maintained. Finally, we did not determine MDA at peak exercise. This is important because patients with chronic HF have an increase in MDA plasma levels with exercise, and its change has been inversely correlated with exercise capacity.[8]

In summary, we demonstrated that higher levels of oxidative stress in patients with chronic HF

were correlated with an increased catecholaminergic response to exercise and with a decreased index of chronotropic responsiveness.

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