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Dobutamine Stress Testing as a Diagnostic Tool for Evaluation of Myocardial Contractile Reserve in Asymptomatic or Mildly Symptomatic Patients With Dilated Cardiomyopathy

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OBJECTIVES We performed dobutamine stress testing for evaluation of myocardial contractile reserve in asymptomatic or mildly symptomatic patients with dilated cardiomyopathy (DCM).

BACKGROUND Catecholamine sensitivity is reduced in failing hearts as a result of myocardial abnormalities in the beta-adrenergic receptor signaling pathway. However, little is known about adrenergic myocardial contractile reserve in asymptomatic or mildly symptomatic patients with DCM.

METHODS The maximal first derivative of left ventricular pressure (LV dP/dt_{max}) was determined during infusion of dobutamine (10 $\mu g\ kg^{-1}\ min^{-1}$) in 46 asymptomatic or mildly symptomatic (New York Heart Association functional class I or II) patients with DCM. The expression of messenger ribonucleic acid (mRNA) for contractile regulatory proteins in endomyocardial biopsy specimens was quantified by reverse transcription and real-time polymerase chain reaction analysis. Plasma norepinephrine levels were measured in all patients and [^{123}I]metaiodobenzylguanidine (MIBG) scintigraphy performed.

RESULTS Patients were classified into 3 groups based on the percentage increase in LV dP/dt_{max} induced by dobutamine (ΔLV dP/dt_{max}) and on LV ejection fraction (LVEF) at baseline: group I ($n = 18$): ΔLV $dP/dt_{max} > 100\%$ and LVEF $> 25\%$; group IIa ($n = 17$): ΔLV $dP/dt_{max} \leq 100\%$ and LVEF $> 25\%$; and group IIb ($n = 11$): ΔLV $dP/dt_{max} \leq 100\%$ and LVEF $\leq 25\%$. The amounts of $beta_1$-adrenergic receptor, sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase, and phospholamban mRNA were significantly smaller in groups IIa and IIb than in group I. The plasma norepinephrine level was increased and the delayed heart/mediastinum count ratio in MIBG scintigraphy was decreased in both groups IIa and IIb.

CONCLUSIONS Dobutamine stress testing is a useful diagnostic tool for identifying reduced adrenergic myocardial contractile reserve related to altered myocardial expression of $beta_1$-adrenergic receptor, sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase, and phospholamban genes even in asymptomatic or mildly symptomatic patients with DCM. (J Am Coll Cardiol Img 2008;1:718–26) © 2008 by the American College of Cardiology Foundation

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Dilated cardiomyopathy (DCM) is characterized by progressive left ventricular (LV) dilation and greatly impaired LV systolic function. In spite of progress in pharmacotherapy for end-stage heart failure, the overall prognosis of individuals with DCM is still poor. It is therefore important that DCM patients who are refractory to standard medical treatment be placed under strict management as early as possible.

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Catecholamine sensitivity is reduced in failing hearts as a result of myocardial abnormalities in the beta-adrenergic receptor (AR) signaling pathway, most prominently down-regulation of the $beta_1$-AR (1,2). Dobutamine is a relatively selective $beta_1$-AR agonist with weak $beta_2$- and alpha-AR agonistic activity. Dobutamine stress testing (DST) is performed widely, mostly in patients with coronary artery disease, to assess myocardial viability, and its safety has been well established (3,4). Previous studies have determined myocardial contractile reserve in DCM patients by DST and found that the response to dobutamine is associated with clinical prognosis (5–7). Although the mechanisms responsible for this association have remained unclear, it is possible that adrenergic myocardial contractile reserve revealed by DST reflects molecular biological changes in the myocardium.

We have now evaluated DST as a diagnostic tool for identifying individuals with a reduced adrenergic myocardial contractile reserve among patients with DCM and a New York Heart Association (NYHA) functional class of I or II. We have also examined the relations of such adrenergic myocardial contractile reserve to activity of the sympathetic nervous system and to myocardial expression of genes for contractile regulatory proteins related to beta-AR signaling or intracellular Ca^{2+} handling.

METHODS

Patients. The study protocol was approved by the Ethics Review Board of Nagoya University School of Medicine (approval #359), and written informed consent was obtained from all study participants. We studied 46 DCM patients with a NYHA functional class of I or II. Dilated cardiomyopathy was defined by a left ventricular ejection fraction (LVEF) of <50% (as determined by contrast ventriculography) in the absence of coronary artery stenosis of >50% (as determined by coronary angiography), valvular heart disease, arterial hypertension, and cardiac muscle disease secondary to any known systemic condition (8). Endomyocardial biopsy at the LV posterior wall was performed to exclude myocarditis or specific heart muscle disease. All patients were in normal sinus rhythm.

The patients were hospitalized for examinations and underwent laboratory measurements including neurohumoral factors, echocardiography, resting myocardial [^{123}I]metaiodobenzylguanidine (MIBG) scintigraphy, and cardiac catheterization. Fourteen patients had been treated with beta-blockers, 11 with digitalis, 34 with diuretics, and 33 with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers (or both). All medications were discontinued at least 4 days before the study. Sixteen patients had been hospitalized because of heart failure with dyspnea on exertion or peripheral edema, but they had been in a stable condition for at least 3 months and were classified as NYHA functional class II at the time of hospitalization for the study.

Myocardial [^{123}I]MIBG scintigraphy. Myocardial [^{123}I]MIBG scintigraphic imaging was performed as previously described (9). A dose of 148 MBq of [^{123}I]MIBG was injected intravenously with the patient in a supine position. Myocardial [^{123}I]MIBG uptake was quantified in anterior planar views at 15 min (early image) and 4 h (delayed image) after tracer injection. The heart/mediastinum count ratio was determined from the delayed anterior planar [^{123}I]MIBG image. The washout rate was calculated with the following formula: $100\% \times [(H - M)_{early} - (H - M)_{delayed}]/(H - M)_{early}$, where H is the mean counts per pixel in the left ventricle and M is the mean counts per pixel in the upper mediastinum. In our laboratory, the normal range for the delayed heart/mediastinum count ratio is 1.8 to 2.7 and that for the washout rate is 16% to 27%.

Cardiac catheterization. All patients initially underwent routine diagnostic left and right heart catheterization by the femoral approach. A 6-F fluid-filled pigtail catheter with a high-fidelity micromanometer (CA-61000-PLB Pressure-tip Catheter, CD Leycom, Zoetermeer, the Netherlands) was placed in the LV cavity for measurement of LV pressure. We evaluate the maximal first derivative of LV pressure (LV dP/dt_{max}) as an index

ABBREVIATIONS AND ACRONYMS

AR = adrenergic receptor
DCM = dilated cardiomyopathy
DST = dobutamine stress testing
GRK2 = G-protein–coupled receptor kinase 2
LV = left ventricular
LV dP/dt_{max} = maximal first derivative of left ventricular pressure
LVEF = left ventricular ejection fraction
MIBG = metaiodobenzylguanidine
mRNA = messenger ribonucleic acid
NYHA = New York Heart Association
SERCA2a = sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase 2a

of contractility and the pressure half-time ($T_{1/2}$) as an index of isovolumic relaxation as previously described (10). After collection of baseline hemodynamic data, dobutamine was infused intravenously at incremental doses of 5 and 10 μg kg^{-1} min^{-1}, and hemodynamic measurements were made at the end of each 10-min infusion period. After hemodynamic values had returned to baseline, endomyocardial biopsy was performed. Several (at least 3) endomyocardial biopsy specimens for messenger ribonucleic acid (mRNA) analysis were frozen immediately in liquid nitrogen and stored at −80°C until the analysis.

Quantitative reverse transcription–polymerase chain reaction analysis. Total RNA was isolated from 1 to 2.5 mg of frozen LV biopsy specimens and subjected to quantitative reverse transcription and polymerase chain reaction analysis, as previously described (10,11), with primers and TaqMan probes (Nippon EGT Co. Ltd., Toyama, Japan) specific for human complementary DNA encoding beta$_1$-AR, beta$_2$-AR, sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase 2a (SERCA2a), phospholamban, ryanodine receptor-2, calsequestrin, and the Na^+/Ca^{2+} exchanger. The primer sequences (forward and reverse, respectively) for G-protein–coupled receptor kinase 2 (GRK2) and for the alpha subunits of G_s and G_{i2} (G_s alpha, G_{i2} alpha) were as follows: GRK2, 5′-TGAGAGCGATAAGTTCACACGG-3′ and 5′-CGCTTTTTGTCCAGGCACT-3′; G_s alpha, 5′-GTACTCCCTGGACAAGATCGACG-3′ and 5′-GCAGTCACATCGTTGAAGCACT-3′; and G_{i2}alpha, 5′-GGAATACCAGCTCAACGACTCA-3′ and 5′-CTGACCACCCACATCAAACATCT-3′. The amount of each mRNA was thus determined with a fluorogenic 5′-nuclease polymerase chain reaction assay and an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster City, California). All determinations were performed in triplicate. The abundance of each mRNA was normalized by the corresponding amount of glyceraldehyde-3-phosphate dehydrogenase mRNA.

Statistical analysis. Data are presented as means ± standard deviation. For comparisons among groups, 1-way analysis of variance was used for continuous variables when appropriate and contingency table analysis was used for categorical variables. Given that the plasma concentration of brain natriuretic peptide was not normally distributed, we assessed differences in such values with the nonparametric Kruskal-Wallis test. When a significant difference was detected, intergroup comparisons were performed with Bonferroni's multiple comparison test. A p value of <0.05 was considered statistically significant.

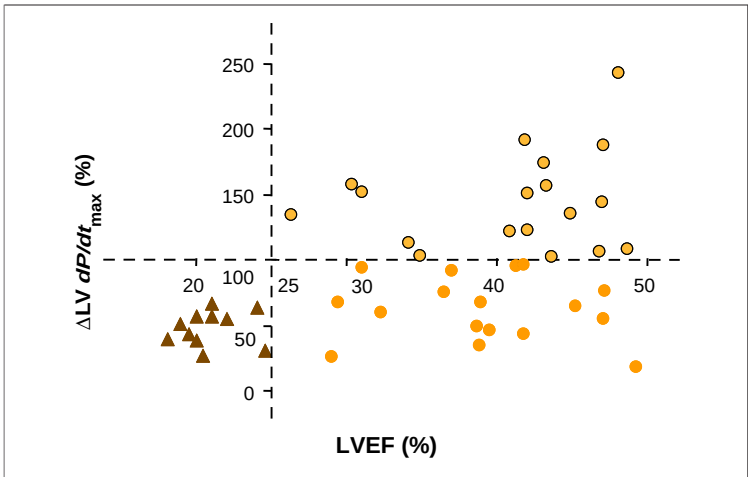

Figure 1. Relation Between Baseline LVEF and ΔLV dP/dt$_{max}$

We calculated the percentage change in maximal first derivative of left ventricular pressure (LV dP/dt$_{max}$ [ΔLV dP/dt$_{max}$]) induced by intravenous infusion of dobutamine (10 μg kg^{-1} min^{-1}). Patients were classified into 3 groups: group I (**orange with black circles**, n = 18), ΔLV dP/dt$_{max}$ >100% (left ventricular ejection fraction [LVEF] >25%); group IIa (**orange circles**, n = 17), ΔLV dP/dt$_{max}$ ≤100% and LVEF >25%; and group IIb (**brown triangles**, n = 11), ΔLV dP/dt$_{max}$ ≤100% and LVEF ≤25%.

RESULTS

Patient classification. Patients were divided into groups on the basis of the adrenergic myocardial contractile response revealed by DST, specifically the percentage change in LV dP/dt$_{max}$ during dobutamine infusion at a dose of 10 μg kg^{-1} min^{-1} relative to the baseline value. Those who showed an increase of >100% were assigned to group I (n = 18), and those who showed an increase of ≤100% were assigned to group II (n = 28). In addition, the patients in group II were further assigned to 2 subgroups according to the LVEF at baseline: those with an LVEF of >25% (group IIa, n = 17) and those with an LVEF of ≤25% (group IIb, n = 11). All of the patients in group I had an LVEF of >25% at baseline (Fig. 1).

Baseline data. Baseline clinical characteristics of the patients are shown in Table 1. The LVEF was significantly decreased in group IIb compared with that in group I or in group IIa, which is consistent with the criterion for subgroup classification. The plasma concentration of brain natriuretic peptide was significantly higher in group IIb than in either of the other 2 groups. Although there were no significant differences in the plasma concentration of this peptide or in resting cardiac function as evaluated by echocardiography or cardiac catheter-

ization between groups I and IIa, the plasma norepinephrine level was significantly higher (Fig. 2) and the delayed heart/mediastinum count ratio was significantly lower (Fig. 3) in group IIa than in group I.

Hemodynamic response to intravenous dobutamine. No complications occurred in any of the study subjects during the dobutamine stress protocol. The effects of intravenous dobutamine infusion on hemodynamics are shown in Table 2. The heart rate at baseline was significantly higher in group IIb than in group I, although that at a dobutamine infusion rate of 10 $\mu g\ kg^{-1}\ min^{-1}$ was similar among the 3 groups. There were no significant differences in LV dP/dt_{max} or $T_{1/2}$ among the 3 groups at baseline. However, LV dP/dt_{max} was significantly higher in group I than in group IIa or in group IIb at dobutamine infusion rates of 5 or 10 $\mu g\ kg^{-1}\ min^{-1}$, and $T_{1/2}$ was significantly greater in group IIb than in group I at a dobutamine infusion rate of 10 $\mu g\ kg^{-1}\ min^{-1}$.

Myocardial [^{123}I]MIBG scintigraphy and the changes in LV dP/dt_{max} during dobutamine infusion of 2 typical cases are presented in Figures 4 and 5.

Expression of contractile regulatory protein genes. The amount of $beta_1$-AR mRNA was significantly reduced in group IIa and in group IIb compared with that in group I (Table 3). There were no significant differences in the expression of $beta_2$-AR, GRK2, G_s alpha, and G_{i2} alpha genes among the 3 groups. With regard to intracellular Ca^{2+}-handling proteins, the abundance of SERCA2a and phospholamban mRNAs was significantly reduced in group IIa and in group IIb compared with that in group I. The abundance of ryanodine receptor-2, calsequestrin, and Na^+/Ca^{2+} exchanger mRNAs did not differ significantly among the 3 groups.

Table 1. Characteristics of the 3 Patient Groups at Baseline

Characteristic	Group I (n = 18)	Group IIa (n = 17)	Group IIb (n = 11)
Age (yrs)	51 ± 9	52 ± 13	50 ± 15
Gender, male/female	12/6	13/4	9/2
NYHA functional class I/II	10/8	8/9	2/9
Medication, n (%)			
Digitalis	3 (17%)	3 (18%)	5 (45%)
Diuretics	10 (56%)	13 (76%)	11 (100%)*
ACE inhibitors or ARBs	13 (72%)	14 (82%)	6 (55%)
Beta-blockers	4 (22%)	5 (29%)	5 (45%)
Spironolactone	6 (33%)	6 (35%)	7 (64%)
LV end-diastolic dimension (mm)	59 ± 4	60 ± 9	72 ± 13*†
LV end-systolic dimension (mm)	47 ± 5	50 ± 10	65 ± 13*†
IVS thickness (mm)	9 ± 1	9 ± 2	8 ± 2
LVPW thickness (mm)	9 ± 1	9 ± 2	9 ± 2
LVEF (%)	41 ± 7	39 ± 6	21 ± 2*†
LV end-diastolic pressure (mm Hg)	17 ± 5	15 ± 7	18 ± 9
PAWP (mm Hg)	11 ± 4	11 ± 4	17 ± 8*†
Cardiac index ($l\ min^{-1}\ m^{-2}$)	3.1 ± 0.5	3.0 ± 0.7	2.7 ± 0.5
Plasma BNP (pg/ml)	59 ± 76	140 ± 147	421 ± 442*†
Plasma norepinephrine (pg/ml)	403 ± 143	693 ± 293*	655 ± 267*
Delayed H/M ratio	1.9 ± 0.2	1.6 ± 0.3*	1.5 ± 0.3*
Washout rate (%)	24.7 ± 12.6	35.6 ± 16.6	42.8 ± 15.4*

Data are means ± SD. *$p < 0.05$ versus group I; †$p < 0.05$ versus group IIa.
ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blocker; BNP = brain natriuretic peptide; H/M = heart/mediastinum; IVS = interventricular septum; LV = left ventricular; LVEF = left ventricular ejection fraction; LVPW = left ventricular posterior wall; NYHA = New York Heart Association; PAWP = pulmonary arterial wedge pressure.

DISCUSSION

The main findings of the present study include the following: 1) All patients with severe LV systolic dysfunction (LVEF ≤25%) showed a reduced adrenergic myocardial contractile reserve. 2) In some patients with mild to moderate LV systolic dysfunction (25 < LVEF <50%), adrenergic myocardial contractile reserve was preserved, whereas in others it was reduced. 3) The abundance of mRNA not only for the $beta_1$-AR but also for SERCA2a and phospholamban was reduced in patients with reduced adrenergic myocardial contractile reserve, even in those in whom LV systolic dysfunction was only mild to moderate, like that in the comparison group.

Evaluation of myocardial contractile reserve. Determinants of myocardial contractile reserve include

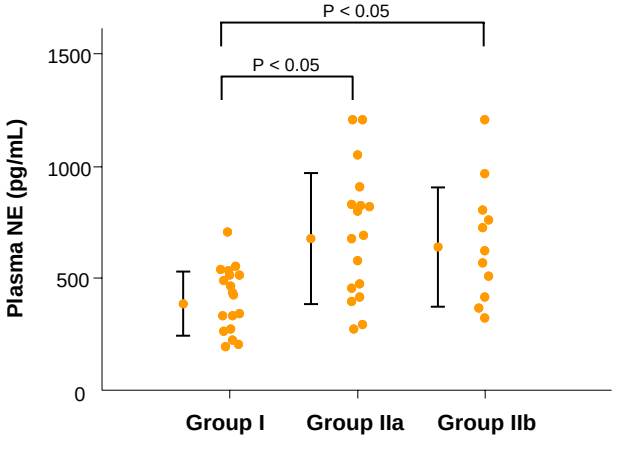

Figure 2. Comparison of Plasma NE Levels Among the 3 Patient Groups

Plasma norepinephrine (NE) levels measured at baseline were significantly higher in group IIa and in group IIb compared with that in group I. Data for individual subjects and the corresponding mean ± SD values are shown.

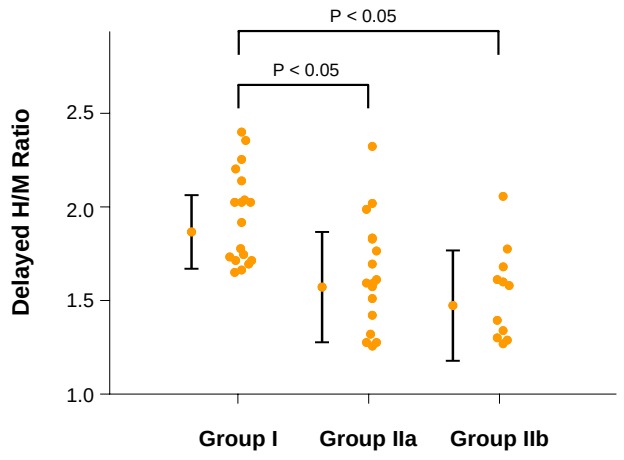

Figure 3. Comparison of the Delayed H/M Ratio in [^{123}I]MIBG Scintigraphy Among the 3 Patient Groups

We determined the heart/mediastinum (H/M) count ratio from the delayed planer [^{123}I]metaiodobenzylguanidine (MIBG) image. The delayed H/M ratio was significantly lower in group IIa and in group IIb compared with that in group I. Data for individual subjects and the corresponding mean ± SD values are shown.

the Frank-Starling mechanism, the force-frequency effect, and adrenergic stimulation (12,13). The myocardial contractile response to adrenergic stimulation is impaired in DCM. Several studies based on dobutamine stress echocardiography have demonstrated a relation between prognosis and adrenergic myocardial contractile reserve, as determined by measurement of indices of LV systolic function such as LVEF or cardiac output (5,6). However, these indices are relatively load-dependent. Few studies have evaluated the adrenergic contractile response to dobutamine infusion by measurement of the increase in LV dP/dt_{max} in patients with nonischemic LV systolic dysfunction (7,14). These studies applied dobutamine stress with atrial pacing or intracoronary dobutamine infusion to avoid the effects of a change in heart rate. In the present study, we evaluated adrenergic myocardial contractile reserve on the basis of the percentage change in LV dP/dt_{max} at an intravenous dobutamine infusion rate of 10 $\mu g\ kg^{-1}\ min^{-1}$, with a cutoff of 100% based on the median value for patients with mild to moderate LV systolic dysfunction (25 < LVEF <50%). Differences in the increase in heart rate among the 3 groups may have influenced the change in LV dP/dt_{max}; patient classification in our study was therefore based on the frequency-dependent inotropic response to dobutamine infusion, which is clinically more useful as a measure of adrenergic myocardial functional reserve.

Table 2. Hemodynamic Response of the 3 Patient Groups to Intravenous Dobutamine Infusion

Parameter	Group I	Group IIa	Group IIb
Heart rate (beats/min)			
Baseline	69 ± 7	76 ± 12	86 ± 13*
Dobutamine (5 $\mu g\ kg^{-1}\ min^{-1}$)	75 ± 10	82 ± 16	90 ± 13*
Dobutamine (10 $\mu g\ kg^{-1}\ min^{-1}$)	98 ± 14	98 ± 20	99 ± 16
LVSP (mm Hg)			
Baseline	122 ± 15	113 ± 16	112 ± 26
Dobutamine (5 $\mu g\ kg^{-1}\ min^{-1}$)	137 ± 21	123 ± 17*	115 ± 23*
Dobutamine (10 $\mu g\ kg^{-1}\ min^{-1}$)	133 ± 16	123 ± 16	124 ± 27
LV dP/dt_{max} (mm Hg/s)			
Baseline	1131 ± 178	1053 ± 190	1048 ± 224
Dobutamine (5 $\mu g\ kg^{-1}\ min^{-1}$)	1746 ± 298	1340 ± 246*	1193 ± 247*
Dobutamine (10 $\mu g\ kg^{-1}\ min^{-1}$)	2741 ± 417	1692 ± 300*	1544 ± 342*
$T_{1/2}$ (ms)			
Baseline	41 ± 6	43 ± 8	44 ± 5
Dobutamine (5 $\mu g\ kg^{-1}\ min^{-1}$)	35 ± 8	37 ± 10	41 ± 5
Dobutamine (10 $\mu g\ kg^{-1}\ min^{-1}$)	25 ± 5	29 ± 11	34 ± 7*

Data are means ± SD. *p < 0.05 versus group I.
LV dP/dt_{max} = maximal first derivative of left ventricular pressure; LVSP = left ventricular systolic pressure; $T_{1/2}$ = pressure half-time.

End-systolic wall stress as assessed by echocardiography has been shown to be a quantitative index of true myocardial afterload and an important determinant of systolic function independent of loading conditions (15). Twenty-three of the 46 patients in the present study (6, 10, and 7 patients in groups I, IIa, and IIb, respectively) underwent echocardiography during DST. The percentage increase in end-systolic wall stress determined by echocardiography during dobutamine infusion tended to be higher in group I than in group IIa or in group IIb (data not shown).

Relation of altered expression of contractile regulatory protein genes to adrenergic myocardial contractile reserve. The failing human heart is characterized by a reduced sensitivity to beta-AR agonists, which may contribute to the loss of cardiac contractility (1,2,14). The decrease in cardiac responsiveness to $beta_1$-AR stimulation appears to be due in part to a reduction in the number of $beta_1$-ARs (14), which in turn is thought to result from a reduction in the amount of the corresponding mRNA (16,17). Our results now suggest that evaluation of adrenergic myocardial contractile reserve by DST might be a means of identifying down-regulation of the $beta_1$-AR in patients with DCM. On the other hand, we did not detect changes in the abundance of GRK2 or G_{i2}alpha mRNAs in patients with a reduced adrenergic myocardial contractile reserve, although the expression of GRK2 and G_{i2}alpha

genes was previously found to be increased in the heart of patients with end-stage heart failure (17,18). This apparent discrepancy might be explained by differences in the patient populations of the studies, with our study including a less severely afflicted ambulatory population of patients with a NYHA functional class of I or II.

The amounts of SERCA2a and phospholamban mRNAs were found to be decreased in endomyocardial biopsy specimens from patients undergoing heart transplantation (19–21). We previously showed that a reduction in the myocardial abundance of SERCA2a mRNA was apparent in patients with impaired myocardial contractile reserve, as revealed by determination of the force-frequency relation, before the onset of detectable LV systolic dysfunction (10). As far as we are aware, however, the relation between adrenergic myocardial contractile reserve and the abundance of mRNAs for Ca^{2+}-handling proteins has not previously been examined. We have now shown that a reduction in adrenergic myocardial contractile reserve appears to be associated with down-regulation not only of $beta_1$-AR mRNA but also of SERCA2a and phospholamban mRNAs, even in asymptomatic or mildly symptomatic patients with DCM. On the other hand, we did not detect changes in the abundance of ryanodine receptor-2, calsequestrin, or Na^+/Ca^{2+} exchanger mRNA in patients with a reduced adrenergic myocardial contractile reserve, which may be considered consistent with previous findings (21–23).

Plasma norepinephrine level and quantitative [^{123}I]MIBG scintigraphy parameters. In heart failure, increased cardiac spillover of norepinephrine and depletion of myocardial catecholamine result in an increased plasma concentration of norepinephrine (24) and decreased cardiac uptake of [^{123}I]MIBG, an analog of norepinephrine (25). In addition, both the plasma norepinephrine level (26) and [^{123}I]MIBG imaging (27) have been found to provide prognostic information in patients with heart failure. We previously showed that decreased [^{123}I]MIBG uptake in DCM patients was associated with a reduced myocardial contractile reserve as evaluated by atrial pacing (9). In the present study, all patients with a greatly increased plasma level of norepinephrine and a greatly reduced [^{123}I]MIBG uptake had a reduced adrenergic myocardial contractile reserve. However, there was marked overlap in moderately increased levels of plasma norepinephrine or moderately reduced levels of [^{123}I]MIBG uptake among patient groups with reduced or preserved adrenergic myocardial contractile reserve. These findings suggest that these resting parameters alone do not fully reflect adrenergic myocardial contractile reserve.

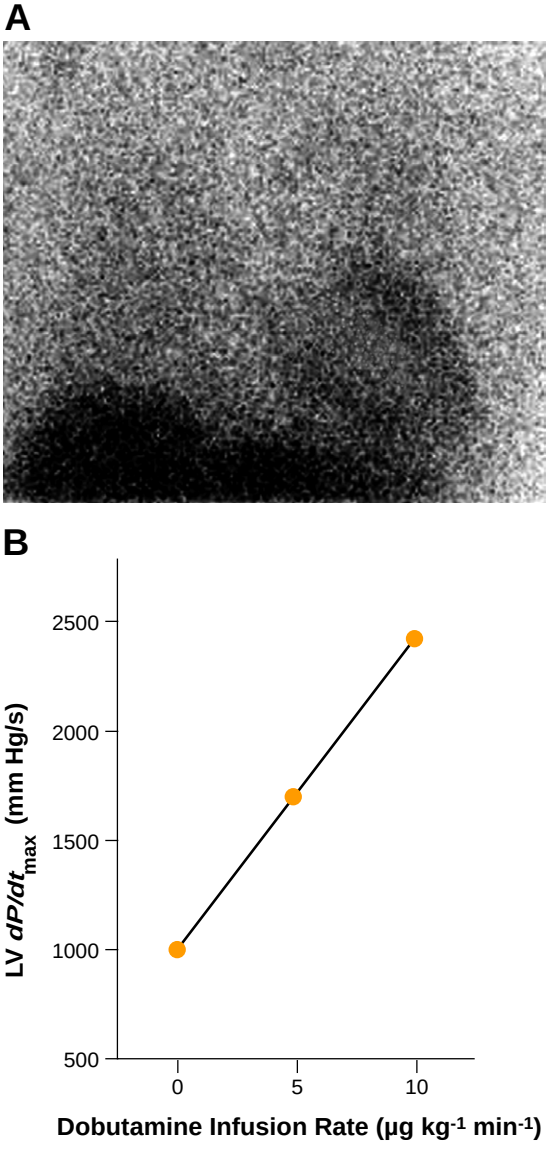

Figure 4. A Representative Case of Group I

This patient was a 43-year-old woman in New York Heart Association functional class I, and she had an LVEF of 43% and a plasma norepinephrine level of 328 pg/ml. **(A)** Mildly reduced myocardial uptake was observed, and the delayed H/M ratio was 2.2. **(B)** The relationship between dobutamine infusion rate and LV dP/dt$_{max}$ of this patient is shown. The LV dP/dt$_{max}$ at baseline and at a dobutamine infusion rate of 10 μg kg^{-1} min^{-1} were 986 and 2,415 mm Hg/s, respectively (ΔLV dP/dt$_{max}$ = 145%). Abbreviations as in Figures 1 and 3.

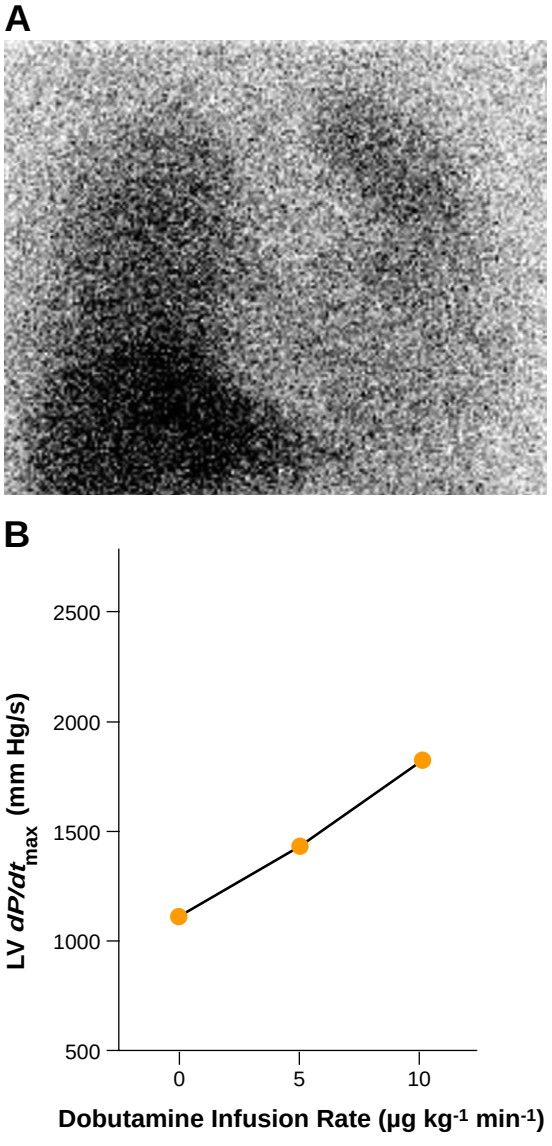

Figure 5. A Representative Case of Group IIa

This patient was a 58-year-old woman in New York Heart Association functional class II, and she had an LVEF of 42% and a plasma norepinephrine level of 1,200 pg/ml. **(A)** Increased lung uptake and severely reduced myocardial uptake are observed, and the delayed H/M ratio was 1.3. **(B)** The relationship between dobutamine infusion rate and LV dP/dt$_{max}$ of this patient is shown. The LV dP/dt$_{max}$ at baseline and at a dobutamine infusion rate of 10 μg kg^{-1} min^{-1} were 1,089 and 1,791 mm Hg/s, respectively (ΔLV dP/dt$_{max}$ = 64%). Abbreviations as in Figures 1 and 3.

Clinical implications. We have evaluated adrenergic myocardial contractile reserve as revealed by DST in DCM patients with a NYHA functional class of I or II. Even among such asymptomatic or mildly symptomatic patients, those individuals with a reduced adrenergic myocardial contractile reserve also manifested activation of the sympathetic nervous system as well as altered myocardial expression of the genes for the beta$_1$-AR, SERCA2a, and phospholamban. Our results suggest that DST is able to identify functional and molecular remodeling associated with overactivation of the sympathetic nervous system in asymptomatic or mildly symptomatic patients with DCM, even when resting parameters still show normal values.

Pharmacological agents that reduce adrenergic hyperactivity, such as beta-blockers or angiotensin-converting enzyme inhibitors, may improve adrenergic myocardial contractile reserve, given that they have been shown to induce up-regulation of myocardial beta-ARs or recovery of the cardiac adrenergic nervous system (28–30). Detrimental changes in cardiac gene expression in patients with DCM have also been shown to be reversed by treatment with beta-blockers (31,32). Although further studies will be necessary to clarify whether abnormalities of intracellular signal transduction related to myocardial contractility influence the prognosis of asymptomatic or mildly symptomatic patients with DCM, DST may provide valuable information for management of these patients.

Study limitations. Concomitant medical treatment may have had an impact on the myocardial abundance of mRNA, although the percentage of patients who had been treated with beta-blockers did not differ significantly among the 3 patient groups. However, given that long-term withholding of medical treatment may be harmful to such individuals, drug treatment was discontinued for only 4 days before the study. We were not able to examine healthy subjects for ethical reasons. In addition, it is difficult to analyze protein abundance and function in the small amounts of tissue obtained by percutaneous endomyocardial biopsy. We therefore assessed contractile regulatory protein expression only by reverse transcription–polymerase chain reaction analysis and exclusively in asymptomatic or mildly symptomatic patients with DCM. Further evaluation of patients with a wider range of heart failure severity may provide insight into the chronology of changes in the expression of contractile regulatory proteins. It remains to be determined whether adrenergic myocardial contractile reserve as assessed by noninvasive diagnostic tools such as dobutamine stress echocardiography is also associated with molecular remodeling of contractile regulatory proteins. Finally, prognostic and therapeutic rel-

evance of DST in DCM remains unclear and deserves further investigation.

CONCLUSIONS

The present study suggests that DST is a useful diagnostic tool for identifying reduced adrenergic myocardial contractile reserve related to altered myocardial expression of the beta$_1$-AR, SERCA2a, and phospholamban genes even in asymptomatic or mildly symptomatic patients with DCM.

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Table 3. Relative Abundance of Contractile Regulatory Protein mRNAs in Endomyocardial Biopsy Specimens Relative to the Corresponding Amount of Glyceraldehyde-3-Phosphate Dehydrogenase mRNA

mRNA	Group I	Group IIa	Group IIb
Beta$_1$-AR	1.39 ± 0.68	0.71 ± 0.19*	0.66 ± 0.29*
Beta$_2$-AR	1.29 ± 0.92	0.95 ± 0.18	0.91 ± 0.40
GRK2	1.54 ± 0.63	1.53 ± 0.26	1.59 ± 0.58
G_s alpha	1.18 ± 0.40	0.94 ± 0.17	1.04 ± 0.34
G_{i2} alpha	0.78 ± 0.35	0.77 ± 0.15	0.85 ± 0.25
SERCA2a	0.60 ± 0.29	0.36 ± 0.08*	0.37 ± 0.12*
Phospholamban	0.82 ± 0.28	0.56 ± 0.12*	0.36 ± 0.16*
Ryanodine receptor-2	0.74 ± 0.42	0.56 ± 0.17	0.69 ± 0.23
Calsequestrin	1.34 ± 0.58	1.16 ± 0.25	1.30 ± 0.44
Na^+/Ca^{2+} exchanger	1.69 ± 0.76	1.14 ± 0.14	1.46 ± 0.84

Data are means ± SD. *p < 0.05 vs. group I.
AR = adrenergic receptor; GRK2 = G protein-coupled receptor kinase 2; mRNA = messenger ribonucleic acid; SERCA2a = sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase 2a.

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Key Words: cardiomyopathy ■ contractility ■ heart failure ■ biopsy ■ dobutamine.

▶APPENDIX

For an accompanying slide set, please see the online version of this article.