

## MYOCARDIAL GENE EXPRESSION IN DILATED CARDIOMYOPATHY TREATED WITH BETA-BLOCKING AGENTS

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### ABSTRACT

**Background** Beta-blocker therapy may improve cardiac function in patients with idiopathic dilated cardiomyopathy. We tested the hypothesis that beta-blocker therapy produces favorable functional effects in dilated cardiomyopathy by altering the expression of myocardial genes that regulate contractility and pathologic hypertrophy.

**Methods** We randomly assigned 53 patients with idiopathic dilated cardiomyopathy to treatment with a  $\beta$ -adrenergic-receptor blocking agent (metoprolol or carvedilol) or placebo. The amount of messenger RNA (mRNA) for contractility-regulating genes (those encoding  $\beta_1$ - and  $\beta_2$ -adrenergic receptors, calcium ATPase in the sarcoplasmic reticulum, and  $\alpha$ - and  $\beta$ -myosin heavy-chain isoforms) and of genes associated with pathologic hypertrophy ( $\beta$ -myosin heavy chain and atrial natriuretic peptide) was measured with a quantitative reverse-transcription polymerase chain reaction in total RNA extracted from biopsy specimens of the right ventricular septal endomyocardium. Myocardial levels of  $\beta$ -adrenergic receptors were also measured. Measurements were conducted at base line and after six months of treatment, and changes in gene expression were compared with changes in the left ventricular ejection fraction as measured by radionuclide ventriculography.

**Results** Twenty-six of 32 beta-blocker-treated patients (those with complete mRNA measurements) had an improvement in left ventricular ejection fraction of at least 5 ejection-fraction (EF) units (mean [ $\pm$ SE] increase,  $18.8 \pm 1.8$ ). As compared with the six beta-blocker-treated patients who did not have a response (mean change, a decrease of  $2.5 \pm 1.8$  EF units), those who did have a response had an increase in sarcoplasmic-reticulum calcium ATPase mRNA and  $\alpha$ -myosin heavy chain mRNA and a decrease in  $\beta$ -myosin heavy chain mRNA. The change in sarcoplasmic-reticulum calcium ATPase was not present in the patients in the placebo group who had a spontaneous response. There were no differences between those who had a response and those who did not in terms of the change in mRNA or protein expression of  $\beta$ -adrenergic receptors.

**Conclusions** In idiopathic dilated cardiomyopathy, functional improvement related to treatment with beta-blockers is associated with changes in myocardial gene expression. (N Engl J Med 2002;346:1357-65.)

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**I**N dilated cardiomyopathy,  $\beta$ -adrenergic-receptor blocking agents improve systolic function and reverse cardiac remodeling by a process that may result from changes in gene expression.<sup>1</sup> Several categories of contractility- and hypertrophy-modifying genes may be involved in producing structural and functional changes in dilated cardiomyopathy (which is characterized by increases in end-systolic and end-diastolic volumes along with decreases in systolic function)<sup>2</sup>; changes prompted by such genes include alterations in the expression of  $\beta$ -adrenergic receptors, calcium-handling proteins, and myosin heavy-chain isoforms.<sup>3</sup> These categories of genes would therefore be candidates for favorable modification by beta-blocking agents.

It is now possible to quantify the levels of messenger RNA (mRNA) in RNA extracted from endomyocardial-biopsy specimens.<sup>4-7</sup> Using this approach and measurements of  $\beta$ -adrenergic-receptor protein,<sup>7</sup> we tested the hypothesis that functional improvement in dilated cardiomyopathy due to beta-blocker therapy,<sup>1,8</sup> including potential quantitative differences in the response to individual agents,<sup>9,10</sup> is related to favorable changes in the expression of genes that regulate contractile function and pathologic hypertrophy.

### METHODS

#### Clinical Protocol

The study was conducted between July 1993 and April 2000. Patients of either sex between 18 and 80 years of age who had chronic symptomatic heart failure due to idiopathic dilated cardiomyopathy and a clinical indication for endomyocardial biopsy were eligible for enrollment. At our two institutions, endomyocardial biopsy is performed routinely in patients with idiopathic dilated cardiomyopathy to rule out inflammation or other infiltrative processes. To be eligible for randomization, patients could not have a lymphocytic infiltrate or other infiltrate in the biopsy specimen, and they had to have a base-line left ventricular ejection fraction, as measured by radionuclide ventriculography, of no more than 0.35 and an increase in the size of the left ventricle, as assessed by two-dimensional echocardiography. Mandatory background therapy consisted of an angiotensin-converting-enzyme inhibitor and digoxin, with diuretics as needed.

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Eligible patients gave their written informed consent on forms that had been approved by the institutional review boards of the University of Colorado Health Sciences Center or University of Utah Health Sciences Center. They then underwent base-line tests designed to measure the expression of myocardial genes, left and right ventricular function, hemodynamics, and the level of cardiac adrenergic activity as estimated by coronary sinus norepinephrine levels. The patients were then randomly assigned in a double-blind manner to treatment with placebo, metoprolol tartrate, or carvedilol. The initial dose of carvedilol was 3.125 mg twice daily, and the initial dose of metoprolol was 6.25 mg twice daily. The dose of study medication was doubled weekly until target doses were reached or until limiting side effects occurred. Target doses were 50 mg of carvedilol twice daily and 100 mg of metoprolol twice daily for patients weighing 85 kg or more and 25 mg of carvedilol twice daily and 50 mg of metoprolol twice daily for patients weighing less than 85 kg. After six months of treatment, all the tests performed at base line were repeated.

Random assignment to placebo was stopped on January 13, 1998, when the estimated sample size had been reached for the detection of differences in  $\beta$ -adrenergic-receptor mRNA expression and protein levels; 44 patients had been enrolled. Nine additional patients were then enrolled and randomly assigned to treatment with metoprolol or carvedilol. In these additional patients, the analysis of mRNA expression was expanded to include gene-chip technology,<sup>11</sup> which took the place of measurement of  $\beta$ -adrenergic-receptor proteins. A total of 53 patients participated in the study: 15 were randomly assigned to placebo, 17 to metoprolol, and 21 to carvedilol.

Base-line measurements of mRNA and receptor protein in these 53 patients with idiopathic dilated cardiomyopathy were compared with measurements in 8 patients with normal left ventricular function who underwent endomyocardial biopsy to rule out inflammatory or infiltrative myocardial disease or as part of a chemotherapy protocol.<sup>7</sup>

#### Measurements of Gene Expression and $\beta$ -Adrenergic-Receptor Proteins

Right-sided heart catheterization and biopsy of right ventricular septal endomyocardium were performed from the right internal jugular vein with the use of combined fluoroscopic and echocardiographic guidance, as previously described.<sup>7</sup> Total RNA was extracted from two, three, or four endomyocardial-biopsy specimens, as previously described.<sup>7</sup> In the extracted RNA, the amount of mRNA that encoded  $\beta_1$ - and  $\beta_2$ -adrenergic receptors,  $\alpha$ - and  $\beta$ -myosin heavy chain, atrial natriuretic peptide, and sarcoplasmic reticulum calcium ATPase was measured by the reverse-transcription quantitative polymerase chain reaction with the use of previously described methods and primers for each reverse-transcribed complementary DNA.<sup>7</sup>

The total density of  $\beta$ -adrenergic-receptor and  $\beta_1$ - and  $\beta_2$ -adrenergic-receptor subtypes was measured in biopsy specimens as previously described,<sup>7</sup> after extensive washing (three washes with a buffer composed of 20 mM TRIS, 150 mM sodium chloride, and 1 mM ascorbate at pH 7.8 and 4°C) to remove bound therapeutically administered beta-blockers. Plasma norepinephrine was measured in coronary-sinus and arterial blood by a radioenzymatic method.<sup>12</sup>

#### Measurements of Ejection Fraction

Left ventricular and right ventricular ejection fractions were measured by in vitro red-cell labeling and radionuclide ventriculography, as previously described.<sup>13,14</sup> The results were expressed in terms of ejection-fraction (EF) units, calculated by dividing the stroke volume by the end-diastolic volume and multiplying by 100.

#### Statistical Analysis

The investigators had full access to the data and performed the analyses independently of the sponsor. Ejection fractions were measured at base line and on completion of the six-month study in a total of 49 patients, who were divided into those who had a response (predefined as an increase in left ventricular ejection fraction by at least 5 EF units) during this period and those who did not have such a response. Potential differences within subgroups of patients with a response and subgroups of those without a response were assessed by paired t-tests performed on base-line measurements and end-of-study measurements. Unpaired t-tests and Mann-Whitney tests were used to assess differences between controls and patients with cardiomyopathy, between the placebo group and the combined beta-blocker groups, and between the patients with a response and those without a response. Analysis of variance with the Bonferroni adjustment was used to assess differences among the three treatment groups (placebo, metoprolol, and carvedilol). A test for interaction was used to examine changes in gene expression and  $\beta$ -adrenergic-receptor protein level in patients with a response and those without a response in the combined beta-blocker group as compared with changes in the corresponding subgroups in the placebo group. A test for interaction was also used to assess potential differences between the metoprolol group and the carvedilol group. Contingency-table analysis was used to detect differences in binary variables among treatment groups. All the tests were two-sided, and a P value of less than 0.05 was considered to indicate statistical significance.

## RESULTS

#### Base-Line Characteristics and General Outcomes

Table 1 presents the base-line characteristics of the 49 patients who completed the trial. There were no significant differences among the treatment groups with respect to demographic characteristics, ventricular function, or hemodynamic variables. The patients with dilated cardiomyopathy were relatively young (50 to 56 years), with moderate-to-severe left ventricular dysfunction (left ventricular ejection fraction, 19 to 25 EF units), and had high levels of cardiac adrenergic activity (coronary-sinus norepinephrine level, >800 pg per milliliter). All the patients tolerated the initiation of treatment and the initial increase in the dose of study medication. All 15 of the patients who received placebo, 14 of the 17 patients who received metoprolol, and 20 of the 21 patients who received carvedilol completed the six-month treatment period. Of the patients who received metoprolol, one underwent cardiac transplantation and two withdrew for other reasons. One patient who received carvedilol died suddenly, six weeks after enrollment. The mean ( $\pm$ SD) total daily doses of metoprolol and carvedilol in the groups of patients who received those drugs and who completed the study were  $125 \pm 47$  mg and  $70 \pm 29$  mg, respectively. None of the patients had complications of endomyocardial biopsy or right-sided heart catheterization. No infiltrative or inflammatory processes were identified by light microscopy, which revealed evidence of hypertrophy and variable amounts of interstitial fibrosis in all the patients.

Base-line mRNA expression in the 45 patients

**TABLE 1.** BASE-LINE CHARACTERISTICS OF THE PATIENTS WITH IDIOPATHIC DILATED CARDIOMYOPATHY WHO COMPLETED THE STUDY.\*

VARIABLE	PLACEBO (N=15)	METOPROLOL (N=14)	CARVEDILOL (N=20)
Age (yr)	56±9	53±10	50±14
Sex (no. of patients)			
Female	7	5	8
Male	8	9	12
Left ventricular ejection fraction (EF units)	25.3±9.6	22.2±9.1	18.9±6.0
Right ventricular ejection fraction (EF units)	33.3±11.7	35.7±11.4	30.4±9.0
NYHA functional class (no. of patients)			
II	5	3	2
III	10	11	16
IV	0	0	2
Peak oxygen consumption (ml/kg of body weight/min)	16.2±3.1	15.2±3.9	17.0±5.8
Heart rate (beats/min)			
Peak during exercise	142±11	140±20	146±26
At time of catheterization	88±16	88±17	88±19
Mean arterial pressure at time of catheterization (mm Hg)	86±12	85±15	85±13
Right atrial pressure (mm Hg)	4.6±4.8	5.9±5.2	6.6±4.4
Pulmonary-artery pressure (mm Hg)	21.5±9.9	26.7±8.5	28.2±9.2
Pulmonary-capillary wedge pressure (mm Hg)	11.0±6.6	13.7±7.4	15.6±7.5
Cardiac index (liters/min/m <sup>2</sup> )	2.67±0.55	2.42±0.70	2.31±0.50
Stroke-volume index (ml/beat/m <sup>2</sup> )	31.6±8.3	27.8±8.3	28.0±9.6
Left ventricular stroke-work index (g/m/m <sup>2</sup> )	32.6±12.9	27.9±12.6	27.4±2.6
Norepinephrine level (pg/ml)			
Arterial	752±548	768±602	644±486
Coronary sinus	1188±801	880±658	891±554

\*Plus-minus values are means ±SD. EF denotes ejection fraction, and NYHA New York Heart Association. EF units are calculated by dividing the stroke volume by the diastolic volume and multiplying by 100. There were no significant differences among the three groups of patients in any of the variables.

with idiopathic dilated cardiomyopathy and complete data for this variable are given in Table 2, as are base-line  $\beta$ -adrenergic-receptor protein levels in the 36 patients with complete data for that variable. The patients with idiopathic dilated cardiomyopathy had lower levels of mRNA for  $\beta_1$ -adrenergic-receptor,  $\alpha$ -myosin heavy chain, and sarcoplasmic-reticulum calcium ATPase than the eight control patients, and they had higher levels of atrial natriuretic peptide mRNA and somewhat (but not significantly) higher levels of  $\beta$ -myosin heavy chain mRNA. In addition, the level of  $\beta_1$ -adrenergic-receptor proteins and its proportion of the total level of  $\beta$ -adrenergic-receptor proteins were lower in the patients with idiopathic dilated cardiomyopathy than in the controls, as was (to a marginal degree) the total  $\beta$ -adrenergic-receptor protein level ( $P=0.057$ ). Among the three treatment groups, the only base-line difference in mRNA expression or receptor-protein levels was a lower level of  $\beta_2$ -adrenergic-receptor protein in the carvedilol group ( $15.0\pm6.4$  fmol per milligram)

than in the placebo group ( $27.1\pm14.5$  fmol per milligram,  $P=0.02$ ).

#### Effect of Treatment with Placebo, Metoprolol, or Carvedilol

Of the measurements listed in Tables 1 and 2, there were statistically significant differences among treatment groups in the change in left ventricular ejection fraction, in which both the metoprolol group and the carvedilol group had greater mean ( $\pm$ SE) increases ( $13.9\pm2.5$  and  $15.5\pm2.9$  EF units, respectively) than the placebo group ( $4.2\pm2.8$  EF units,  $P=0.015$  by analysis of variance); the change in left ventricular stroke-work index, in which the carvedilol group had a greater increase than the placebo group ( $11.7\pm3.6$  vs.  $0.0\pm2.6$  ml per beat per square meter of body-surface area,  $P=0.04$ ); and the change in peak heart rate during exercise, in which both beta-blocker groups had greater decreases ( $29.3\pm5.8$  beats per minute in the metoprolol group and  $28.6\pm4.7$  beats per minute in the carvedilol group) than the

**TABLE 2.** BASE-LINE mRNA EXPRESSION AND PROTEIN LEVEL IN THE CONTROL PATIENTS AND THE PATIENTS WITH IDIOPATHIC DILATED CARDIOMYOPATHY.\*

MEASUREMENT	CONTROL PATIENTS (N=8)	PATIENTS WITH IDIOPATHIC DILATED CARDIOMYOPATHY (N=45)	P VALUE
<b>Gene expression</b>			
Age (yr)	49.1±12.9	54.1±10.5	0.24
Sex			0.35
Female	5	20	
Male	3	25	
Left ventricular ejection fraction (EF units)	58.9±9.6	21.4±8.2	0.001
Right ventricular ejection fraction (EF units)	47.6±6.2	33.0±10.6	0.001
$\beta_1$ -Adrenergic receptor (molecules mRNA/ $\mu$ g of total RNA)	305,000±205,000	167,000±158,000	0.034
$\beta_2$ -Adrenergic receptor (molecules mRNA/ $\mu$ g of total RNA)	265,000±151,000	261,000±179,000	0.96
Atrial natriuretic peptide (molecules mRNA/ $\mu$ g of total RNA)	3,400,000±3,270,000	8,150,000±5,510,000	0.022
Sarcoplasmic-reticulum calcium ATPase (molecules mRNA/ $\mu$ g of total RNA)	8,280,000±2,670,000	6,110,000±2,240,000	0.035
Myosin heavy chain			
$\alpha$ -Myosin (molecules mRNA/ $\mu$ g of total RNA)	3,710,000±1,920,000	1,090,000±670,000	0.001
$\beta$ -Myosin (molecules mRNA/ $\mu$ g of total RNA)	11,400,000±3,300,000	16,200,000±6,600,000	0.086
Total (molecules mRNA/ $\mu$ g of total RNA)	14,800,000±3,900,000	17,200,000±6,900,000	0.39
Proportion $\alpha$ -myosin (%)	23.1±8.1	6.5±3.5	0.001
Proportion $\beta$ -myosin (%)	76.9±8.1	93.4±3.5	0.001
	<b>CONTROL PATIENTS (N=8)</b>	<b>PATIENTS WITH IDIOPATHIC DILATED CARDIOMYOPATHY (N=36)</b>	
<b><math>\beta</math>-Adrenergic-receptor protein</b>			
Age (yr)	49.1±12.9	53.8±11.5	0.32
Sex (no. of patients)			0.70
Female	5	17	
Male	3	19	
Left ventricular ejection fraction (EF units)	58.9±9.6	22.0±8.2	<0.001
Right ventricular ejection fraction (EF units)	47.6±6.2	33.4±11.6	0.003
Measured level			
Total (fmol/mg of protein)	74.5±34.6	50.6±30.6	0.057
$\beta_1$ -Adrenergic receptor (fmol/mg of protein)	57.7±29.4	31.0±25.5	0.013
$\beta_2$ -Adrenergic receptor (fmol/mg of protein)	16.8±10.3	19.7±12.8	0.56
Proportion $\beta_1$ -adrenergic receptor (%)	77.0±11.5	58.7±21.1	0.023
Proportion $\beta_2$ -adrenergic receptor (%)	23.0±11.5	41.3±21.1	0.023

\*Plus-minus values are means  $\pm$ SD. Of the 53 patients with idiopathic dilated cardiomyopathy, 45 had complete base-line and six-month data for mRNA expression, and 36 had complete base-line and six-month data for levels of  $\beta$ -adrenergic-receptor proteins. Values for  $\alpha$ -myosin, proportion  $\alpha$ -myosin, and proportion  $\beta$ -myosin were compared by the Mann-Whitney test; all other continuous variables were compared by the unpaired t-test.

placebo group ( $2.8 \pm 5.3$  beats per minute,  $P=0.005$ ). When the beta-blocker groups were combined, changes in these variables were significantly different from changes in the placebo group; there was also a greater reduction in the heart rate at rest in the combined beta-blocker group.

#### Changes in Functional Class, Hemodynamic Variables, Functional Capacity, Ventricular Function, and Coronary-Sinus Norepinephrine

To determine what changes are specifically associated with improvement in left ventricular function

and reversal of remodeling, we divided the placebo, metoprolol, and carvedilol groups into subgroups according to the presence or absence of a response (an increase in left ventricular ejection fraction of at least 5 EF units) after six months of treatment. In the placebo group, there were 5 patients with a response and 10 without a response; in the metoprolol group, there were 12 with a response and 2 without a response; and in the carvedilol group, there were 16 patients with a response and 4 without a response ( $P=0.003$  by contingency-table analysis). In a test for

interaction, changes in left ventricular ejection fraction, hemodynamic variables, and gene expression did not differ among the four response or nonresponse subgroups of patients who were taking metoprolol or carvedilol. In subsequent analyses the patients who had a response with either beta-blocker were combined, as were those who did not have a response with either beta-blocker.

There were differences between patients with a response and those without a response in both the placebo group and the combined beta-blocker groups with respect to left ventricular ejection fraction and right ventricular ejection fraction, but with respect to no other variables (Table 3). According to peak heart rate during exercise, the response and nonresponse subgroups among the patients who received beta-blockers had nearly identical degrees of beta-blockade; their decreases in heart rate during exercise were 28.9 and 28.8 beats per minute, respectively.

Placebo-treated patients who had a response (in terms of left ventricular ejection fraction) also had an improvement in right ventricular ejection fraction, but they had no statistically significant change in any other variable during the six-month study (Table 3). There were no significant changes in the subgroup of placebo-treated patients who did not have a response. Patients in the beta-blocker group who had an improvement in left ventricular ejection fraction also had increases in right ventricular ejection fraction, stroke-volume index, and left ventricular stroke-work index and decreases in New York Heart Association class, heart rate at rest, peak heart rate during exercise, mean pulmonary-artery pressure, and mean pulmonary-capillary wedge pressure. In contrast, the only change in the subgroup of beta-blocker-treated patients who did not have a response was a reduction in peak heart rate during exercise.

#### Changes in Gene Expression and Receptor Density

Figure 1 shows data for changes in the quantity of mRNA and in  $\beta$ -adrenergic-receptor protein levels in the subgroups of patients with or without a response in the placebo group and the combined beta-blocker groups. In the placebo group, there were no significant differences between patients with a response and those without a response in either the amount of any mRNA or the levels of  $\beta$ -adrenergic-receptor proteins. In contrast, among the beta-blocker-treated patients, those with a response had an increase in the amount of  $\alpha$ -myosin heavy chain mRNA and sarcoplasmic-reticulum calcium ATPase mRNA, as well as a decrease in  $\beta$ -myosin heavy chain mRNA (Fig. 1B). In a test for interaction, the difference between the response and nonresponse subgroups in the change in sarcoplasmic-reticulum calcium ATPase mRNA was significant ( $P=0.046$ ), but the changes

in mRNA for myosin heavy-chain isoforms were not significant, in the placebo group as compared with the beta-blocker group. Among the beta-blocker-treated patients, there were no significant differences or trends between those with a response and those without a response with respect to changes in  $\beta_1$ - or  $\beta_2$ -adrenergic-receptor mRNA or protein levels (Fig. 1B and 1D, respectively).

For changes within the subgroups shown in Figure 1, among the small number of patients in the placebo group who had a response, there were no significant changes in mRNA expression or  $\beta$ -adrenergic-receptor protein levels during the treatment period (Fig. 1A and 1C, respectively); the increase in  $\alpha$ -myosin heavy chain mRNA reached a significance level of  $P=0.15$ . Among the patients in the placebo group who did not have a response, there were increases over time in total  $\beta$ -adrenergic-receptor level,  $\beta_1$ -adrenergic-receptor level, and the percentage of  $\beta_1$ -adrenergic receptor (Fig. 1C), and there was a trend toward a decrease in the expression of atrial natriuretic peptide mRNA (Fig. 1A).

Among the patients in the combined beta-blocker groups who had a response, there were increases over time in the amount of mRNA for  $\beta_2$ -adrenergic-receptor, sarcoplasmic-reticulum calcium ATPase, and  $\alpha$ -myosin heavy chain (Fig. 1B) and in total  $\beta$ -adrenergic-receptor and  $\beta_1$ -adrenergic-receptor protein levels (Fig. 1D). The subgroup of beta-blocker-treated patients with a response also had a decrease in the expression of atrial natriuretic peptide mRNA (Fig. 1B). Among the beta-blocker-treated patients without a response, there was a trend toward an increase in the expression of  $\beta$ -myosin heavy chain mRNA and no significant change in any other mRNA or receptor measurement (Fig. 1B and 1D).

#### DISCUSSION

Treatment of patients with dilated cardiomyopathy results in improved intrinsic systolic function (assessed in terms of ejection fraction or more load-independent indexes<sup>1</sup>) and a reversal of cardiac remodeling (assessed in terms of ejection fraction,<sup>1,8,15</sup> ventricular volume<sup>13,14</sup> or dimension,<sup>16-18</sup> or left ventricular mass and chamber shape<sup>17,18</sup>). According to the definition of a "response" as an increase in left ventricular ejection fraction of at least 5 EF units, these favorable changes occur in 50 to 70 percent of beta-blocker-treated patients<sup>19-22</sup> (62 percent in the Multicenter Oral Carvedilol Heart Failure Assessment [MOCHA] Trial<sup>22</sup> [unpublished data]). Use of the left ventricular ejection fraction to measure the response to a beta-blocker allows assessment of both intrinsic systolic function<sup>23</sup> and the degree of cardiac remodeling,<sup>15</sup> the two fundamental physiological processes that characterize dilated cardiomyopathy,<sup>8,24</sup> because the

**TABLE 3.** CHANGES IN VENTRICULAR FUNCTION, HEMODYNAMIC VARIABLES, FUNCTIONAL CAPACITY, AND CARDIAC ADRENERGIC DRIVE DURING THE SIX-MONTH STUDY, ACCORDING TO THE PRESENCE OR ABSENCE OF AN INCREASE IN LEFT VENTRICULAR EJECTION FRACTION WITH TREATMENT.\*

VARIABLE	PLACEBO		BETA-BLOCKER	
	RESPONSE (≥5 EF UNITS) (N=5)	NO RESPONSE (<5 EF UNITS) (N=10)	RESPONSE (≥5 EF UNITS) (N=28)	NO RESPONSE (<5 EF UNITS) (N=6)
NYHA functional class	-0.4±0.5	-0.2±0.1	-0.6±0.1†	-0.5±0.2‡
Left ventricular ejection fraction (% change in EF units)	17.0±3.4†§	-2.2±1.3	18.5±1.7†§	-2.5±1.8
Right ventricular ejection fraction (% change in EF units)	10.4±3.6†§	0.0±1.7	11.4±2.0†§	-1.5±4.2
Peak oxygen consumption (ml/kg/min)	2.7±2.1	1.1±1.3	0.8±0.8	1.2±2.6
Heart rate (beats/min)				
Peak during exercise	-10.3±10.4	1.8±5.8	-28.9±4.2†	-28.8±7.6†
At rest	0.4±2.7	-13.2±7.8	-20.8±2.9†	-13.7±7.0
Mean arterial pressure (mm Hg)	-10.8±4.8‡	-5.9±5.8	0.6±3.2	-3.0±3.7
Right atrial pressure (mm Hg)	-0.4±1.5	2.4±1.6	-1.4±0.9	-2.3±1.1‡
Pulmonary-artery pressure (mm Hg)	-2.6±2.3	-0.4±2.2	-5.9±2.0†	-3.0±4.1
Pulmonary-capillary wedge pressure (mm Hg)	-2.6±1.4	0.8±2.1	-5.0±1.8†	-3.0±2.5
Cardiac index (liters/min/m <sup>2</sup> )	0.14±0.26	-0.2±0.2	0.08±0.14	0.33±0.27
Stroke-volume index (ml/min/m <sup>2</sup> )	0.35±3.4	4.1±2.3	9.9±2.2†	10.1±5.7
Left ventricular stroke-work index (g/m/m <sup>2</sup> )	-4.4±2.0	2.5±3.7	11.8±2.7†	8.2±4.1
Coronary sinus norepinephrine level (pg/ml)	-240±231	-416±193‡	-201±140	67±228

\*Plus-minus values are means ±SE. A minus sign denotes a decrease.

†P<0.05 for the change between the base-line value and the value measured at six months, by the paired t-test.

‡P<0.10 for the change between the base-line value and the value measured at six months, by the paired t-test.

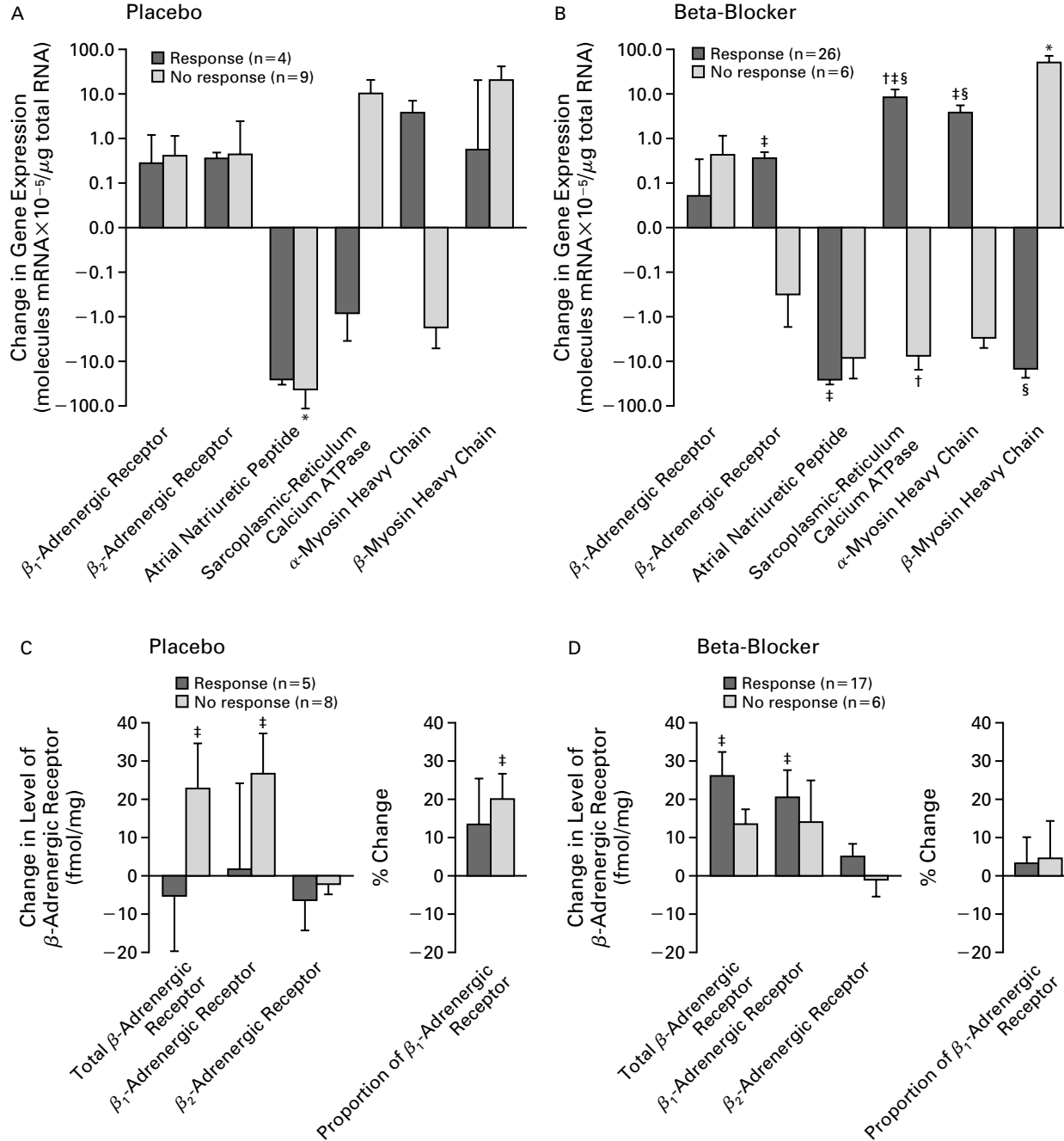
§P<0.05 by the unpaired t-test for the comparison with patients in the same treatment group (placebo or beta-blocker) who did not have a response.

**Figure 1 (facing page).** Changes in Myocardial Messenger RNA (mRNA) Expression and β-Adrenergic-Receptor Protein Levels.

Panels A and B show changes between base line and the end of the six-month study in the abundance of myocardial mRNA for six contractility-regulating or hypertrophy-regulating proteins in patients who received placebo or a beta-blocker. The changes in patients who had an improvement in left ventricular ejection fraction (a "response," defined as an increase by at least 5 ejection fraction [EF] units) were compared with the changes in patients who did not have such a response. Gene expression is shown as molecules of mRNA per microgram of total RNA on a logarithmic scale; I bars represent standard errors, plotted within the unit scales of the nominal absolute changes. Panels C and D show changes in the levels of β-adrenergic-receptor proteins in patients given placebo or a beta-blocker. Changes in patients who had a response were compared with changes in patients who did not have a response. I bars represent standard errors. The asterisk indicates P<0.10 for the change between the base-line value and the value measured at six months, by the paired t-test; the daggers P<0.05 for the comparison with the placebo group by the test for interaction; the double daggers P<0.05 for the change between the base-line value and the value measured at six months, by the paired t-test; and the section marks P<0.05 for the comparison with patients who did not have a response. Each panel shows results for patients with complete data for the indicated mRNA and receptor-protein measurements.

calculation incorporates end-systolic volume in the numerator and end-diastolic volume in the denominator. Moreover, in dilated cardiomyopathy, left ventricular ejection fraction is a major determinant of clinical outcome,<sup>25,26</sup> and improvement in this variable is probably related to the substantial clinical benefit produced by beta-blocking agents.<sup>1,8,27</sup>

We reasoned that time-dependent improvement in idiopathic dilated cardiomyopathy with beta-blocker therapy would be accompanied by changes in myocardial gene expression, defined as the quantity of mRNA or proteins in the steady state. Three different categories of genes involved in the regulation of contractility<sup>3</sup> were assessed:  $\beta$ -adrenergic receptors, sarco-



plasmic-reticulum calcium ATPase (which is involved in calcium handling), and the contractile-protein isoforms  $\alpha$ -myosin and  $\beta$ -myosin heavy chain. In addition, the mRNA expression of one protein directly involved in pathologic hypertrophy ( $\beta$ -myosin heavy chain) and of one molecular marker of hypertrophy (atrial natriuretic peptide) was measured. Gene expression in all three categories of contractility-regulating genes was initially abnormal in the patients with idiopathic dilated cardiomyopathy, as compared with the controls. The investigational strategy was to study changes in gene expression in biopsy specimens from ventricles that showed phenotypic improvement as compared with those that did not show improvement, in beta-blocker-treated as compared with placebo-treated patients. Right ventricular septal specimens were used for gene-expression measurements and were related to changes in left ventricular ejection fraction for three reasons: the ventricular septum is a shared wall; in idiopathic dilated cardiomyopathy, changes in gene expression in the septum reflect those in both the right ventricular and left ventricular free walls<sup>7</sup>; and the left ventricular ejection fraction is more reliably measured than the right ventricular ejection fraction.<sup>28</sup>

The main finding was that in idiopathic dilated cardiomyopathy, improvement in left ventricular ejection fraction by beta-blocking agents is specifically associated with favorable changes in the expression of genes encoding sarcoplasmic-reticulum calcium ATPase and the  $\alpha$  and  $\beta$  isoforms of myosin heavy chain, but not the genes encoding  $\beta$ -adrenergic receptors. The changes in sarcoplasmic-reticulum calcium ATPase and the myosin heavy chains, coupled with a statistically significant reduction in the expression of atrial natriuretic peptide mRNA, indicate that beta-blocker therapy is associated with molecular remodeling in the form of reversal of induction of elements of the "fetal" gene program<sup>29,30</sup> in patients who have a favorable myocardial response to treatment. There were no differences in mean pulmonary capillary-wedge pressure or mean systemic arterial pressure between the beta-blocker-treated patients with a response and those without a response, indicating that regression of the fetal-gene program was not the result of changes in loading conditions.

In terms of the cognate proteins, the increase in the expression of sarcoplasmic-reticulum calcium ATPase and the fast-contracting  $\alpha$ -myosin heavy chain, plus the decrease in the expression of the slow-contracting  $\beta$ -myosin heavy chain, would improve contractile function, and the decreased expression of  $\beta$ -myosin heavy chain would diminish pathologic hypertrophy. However, since fetal-gene proteins were not measured in this study, it is not clear whether the observed changes at the mRNA level led to an improvement in

phenotype. A variety of other gene products capable of influencing contractile function and pathologic hypertrophy were also not measured in this study.

Favorable changes in the expression of  $\alpha$ -myosin heavy chain mRNA tended to occur in the small number of placebo-treated patients with a response as well as in the more numerous beta-blocker-treated patients with a response. In contrast, the up-regulation of mRNA expression of sarcoplasmic-reticulum calcium ATPase in association with improvement in left ventricular function was observed only in the beta-blocker-treated patients who had a response. In experimental models,  $\beta$ -adrenergic stimulation can down-regulate the expression of the gene encoding sarcoplasmic-reticulum calcium ATPase,<sup>31,32</sup> as well as induce the fetal pattern of expression of myosin heavy chain.<sup>32</sup> In view of the high levels of cardiac adrenergic activity present in the study population, we speculate that  $\beta$ -adrenergic stimulation contributed to induction of a pathological fetal gene program, which was reversed by beta-blockade more comprehensively and frequently than by placebo treatment.

In the current study, the degrees of beta-blockade produced by metoprolol (a selective  $\beta_1$ -adrenergic-receptor blocking agent) and carvedilol (which blocks  $\beta_1$ -,  $\beta_2$ -, and  $\alpha_1$ -adrenergic receptors)<sup>8</sup> were substantial and nearly identical, as assessed by a reduction in peak heart rate during exercise. There were no qualitative differences between metoprolol and carvedilol in their effects on any measurement, but a larger sample may be required to detect such differences. In patients with a response in left ventricular ejection fraction, treatment with either drug was associated with increases in the amount of  $\alpha$ -myosin heavy chain and sarcoplasmic-reticulum calcium ATPase mRNA and decreases in  $\beta$ -myosin heavy chain mRNA. These findings probably represent the molecular class effects of  $\beta_1$ -adrenergic-receptor blockade.

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