Cardiac Contractility Modulation Electrical Signals Improve Myocardial Gene Expression in Patients With Heart Failure

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Objectives	The objective of this study was to test whether cardiac contractility modulation (CCM) electric signals induce reverse molecular remodeling in myocardium of patients with heart failure.
Background	Heart failure is associated with up-regulation of myocardial fetal and stretch response genes and down-regulation of Ca^{2+} cycling genes. Treatment with CCM signals has been associated with improved symptoms and exercise tolerance in heart failure patients. We tested the impact of CCM signals on myocardial gene expression in 11 patients.
Methods	Endomyocardial biopsies were obtained at baseline and 3 and 6 months thereafter. The CCM signals were delivered in random order of ON for 3 months and OFF for 3 months. Messenger ribonucleic acid expression was analyzed in the core lab by investigators blinded to treatment sequence. Expression of A- and B-type natriuretic peptides and α-myosin heavy chain (MHC), the sarcoplasmic reticulum genes SERCA-2a, phospholamban and ryanodine receptors, and the stretch response genes p38 mitogen activated protein kinase and p21 Ras were measured using reverse transcription-polymerase chain reaction and bands quantified in densitometric units.
Results	The 3-month therapy OFF phase was associated with increased expression of A- and B-type natriuretic peptides, p38 mitogen activated protein kinase, and p21 Ras and decreased expression of α-MHC, SERCA-2a, phospholamban, and ryanodine receptors. In contrast, the 3-month ON therapy phase resulted in decreased expression of A- and B-type natriuretic peptides, p38 mitogen activated protein kinase and p21 Ras and increased expression of α-MHC, SERCA-2a, phospholamban, and ryanodine receptors.
Conclusions	The CCM signal treatment reverses the cardiac maladaptive fetal gene program and normalizes expression of key sarcoplasmic reticulum Ca^{2+} cycling and stretch response genes. These changes may contribute to the clinical effects of CCM. (J Am Coll Cardiol 2008;51:1784–9) © 2008 by the American College of Cardiology Foundation

A large number of patients with advanced heart failure (HF) are refractory to optimal standard medical therapy. This has given rise to development and testing of a host of new device-based therapies (1). One recent and potentially broadly applicable treatment under investigation is cardiac contractility modulation (CCM) electrical signals (2,3). The CCM signals are relatively high-voltage electrical impulses applied to the myocardium during the absolute refractory period. These signals do not initiate a new contraction or modify activation sequence as is the case with other therapies such as cardiac resynchronization therapy (4). Rather, CCM signals are intended to enhance systolic function of the failing myocardium (5–8). Preliminary clinical studies suggest that CCM signal therapy is safe and can also improve exercise tolerance and quality of life in patients with HF (3,9) without increasing myocardial oxygen consumption (10). Recent studies in experimental HF suggest that a novel mechanism underlying these effects is normalized expression of genes known to be pathologically up- or down-regulated in HF (8). The purpose of this study was to test whether similar effects are present in patients with HF.

Methods

Patients and study protocol. The present study included data from 11 patients. This was a substudy of a random-

ized, double blind, multicenter, crossover study of CCM signal therapy in patients with ejection fraction <35% and New York Heart Association functional class II/III symptomatic HF despite optimal medical therapy conducted at 15 hospitals in Europe and included 164 patients (the FIX-CHF-4 [Fix-Chronic Heart Failure–4] study). The major exclusion criteria included atrial fibrillation, patient eligible for cardiac resynchronization therapy, peak V_{O_2} <10 ml/kg/min, hospitalization within 1 month for acute exacerbation of HF, revascularization within 1 month or acute myocardial infarction within 3 months of study entry.

Qualifying patients were implanted with an Optimizer System (Impulse Dynamics USA, Inc., Orangeburg, New York). Details of the system implant have been provided previously (10). Two weeks following implant, patients were randomized to 3 months of CCM signal treatment (group 1, ON, n = 7) or to sham treatment with the device left off (group 2, OFF, n = 4) (Fig. 1, phase I). Three months later, patients crossed over to the opposite treatment for an additional 3 months (phase II). Protocol-specified clinical follow-up visits at 3 and 6 months included cardiopulmonary stress test (peak V_{O_2}), Minnesota Living with Heart Failure Questionnaire (MLHFQ) and 6-min walk test (6MW). Patients participating in the present substudy underwent right heart biopsies at baseline and at the end of the 3- and 6-month treatment periods. Samples were snap frozen, stored in liquid nitrogen, and sent to a core lab that was blinded to study sequence. Messenger ribonucleic acid (RNA) expression of glyceraldehyde-3-phosphate dehydrogenase, α-myosin heavy chain (MHC), A- and B-type natriuretic proteins, sarcoplasmic reticulum calcium adenosine triphosphatase (SERCA-2a), phospholamban, and ryanodine receptor (RyR2), sodium-calcium exchanger, and the stretch receptor genes p38 mitogen activated protein kinase and p21 Ras were measured. Details of the methods of isolation and analysis are identical to those reported previously (11,12). Band intensity of each gene was expressed as a percent of the intensity of the respective gene at baseline.

Data are expressed as mean ± standard deviation. Data from this crossover study were analyzed according to the methods of Fleiss (13). In brief, after confirming the absence of a treatment-by-period interaction (by repeated measures analysis of variance), mean end-of-period values for ON and OFF periods are pooled and analyzed by paired *t* test. Correlations between clinical outcome data and changes in gene expression were performed by linear regression analysis.

The main study and the substudy were each approved by the local ethics committee. Written informed consent was obtained from all patients prior to entry.

Results

Clinical characteristics. Patient baseline characteristics are summarized in Table 1. All patients were Caucasian men with New York Heart Association functional class III symptoms despite treatment with beta-blockers, angiotensin converting enzyme inhibitors, or angiotensin receptor blockers and, with one exception, spironolactone. Seven of
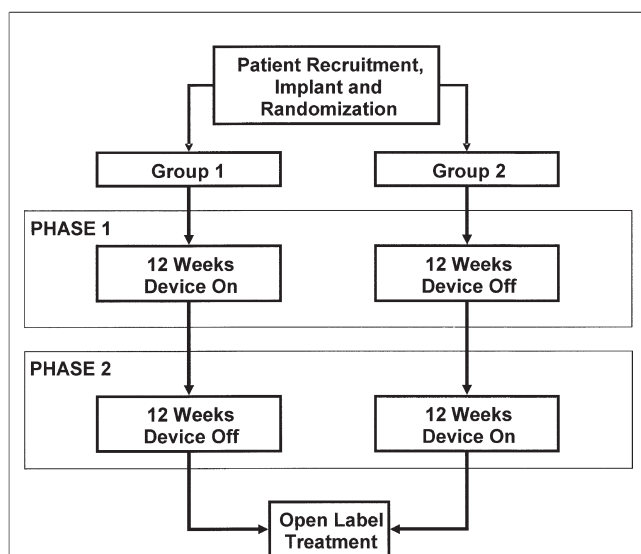
| Figure 1 | FIX-CHF-4 Study Design |

This was a prospective, randomized, double-blind, double crossover study. FIX-CHF-4 = Fix-Chronic Heart Failure–4.

Table 1	Baseline Clinical Characteristics		
	Group 1	**Group 2**	**Overall**
n	7	4	11
Age (yrs)	63 ± 10	63 ± 10	63 ± 9
% male	100	100	100
Idiopathic/ischemic	5/2	2/2	7/4
% NYHA functional class III	100	100	100
EF (%)	26 ± 4	30 ± 7	28 ± 6
QRS (ms)	125 ± 14	122 ± 28	124 ± 19
HR (beats/min)	70 ± 10	80 ± 18	74 ± 13
SBP (mm Hg)	107 ± 11	106 ± 17	107 ± 13
MLHFQ	43 ± 20	28 ± 14	37 ± 19
6MW (m)	458 ± 88	436 ± 85	450 ± 83
Peak V_{O_2} (ml o_2/kg/min)	13.8 ± 2.5	13.2 ± 21	13.6 ± 2.6
dP/dt$_{max}$ (mm Hg/s)	860 ± 94	921 ± 134	881 ± 108
ΔdP/dt$_{max}$ (%)	13 ± 4	12 ± 5	13 ± 4

dP/dt$_{max}$ = peak positive rate of change of left ventricular pressure measured during Optimizer System implant; EF = ejection fraction; HR = heart rate; MLHFQ = Minnesota Living with Heart Failure Questionnaire; NYHA = New York Heart Association functional classification; QRS = QRS duration; SBP = systolic blood pressure; 6MW = 6-min walk test.

the patients were randomized to group 1 (CCM signal treatment during the first 3 months), and 4 patients were randomized to group 2 (placebo during the first 3 months). The groups were reasonably well-balanced with regard to other characteristics.

The MLHFQ scores averaged 23 ± 14 at the end of ON treatment periods compared with 35 ± 17 at the end of OFF treatment periods (p = 0.08). The 6MW averaged 453 ± 97 m at the end of ON treatment periods compared with 402 ± 111 m at the end of OFF treatment periods (p = 0.27). Finally, peak V_{O_2} averaged 14.5 ± 4.3 ml O_2/kg/min at the end of ON treatment periods compared with 11.9 ± 3.8 ml O_2/kg/min at the end of OFF treatment periods (p = 0.15). Because of the small number of patients and large standard deviations, none of these differences was statistically significant.

Gene expression. Typical Northern blots obtained from 1 group 1 and 1 group 2 patient are shown in Figure 2. The consistent intensity of the glyceraldehyde-3-phosphate dehydrogenase band signifies consistent messenger RNA loading between samples. Each panel shows the respective band at baseline, 3 months, and 6 months. Genes that are overexpressed in HF tended to decrease toward normal during CCM signal treatment, whereas genes that are underexpressed in HF tended to increase toward normal during CCM signal treatment. A detailed analysis of the α-MHC blots is shown in Figure 3. Band intensities were

normalized to their respective values obtained in the baseline heart failure state. Data from patients with ischemic and idiopathic cardiomyopathy are shown with dashed and solid lines, respectively. At the end of phase I, α-MHC expression increased in group 1 patients (device ON) (Fig. 3A) and stayed the same or decreased in group 2 patients (device OFF) (Fig. 3B). After crossover, expression decreased in group 1 patients when the device was switched off and increased in group 2 patients when the device was switched on. The overall comparisons are summarized in panel C where results from ON periods are pooled and results from OFF periods are pooled. As shown, there was a statistically significant ~62.7 ± 45.3% increase in α-MHC expression above the HF baseline state in response to CCM signal treatment. As shown in this typical example, there was no substantive difference in the response identified in hearts with idiopathic and ischemic cardiomyopathies.

Results from the other genes examined are summarized in Figure 4. There were statistically significant changes during the ON periods in all genes examined compared to the baseline HF state. The SERCA-2a, phospholamban, and RyR2 (genes whose expression is decreased in chronic HF) all increased in response to 3 months of CCM signal treatment. A- and B-type natriuretic peptides, p38 mitogen activated protein kinase, p21 Ras, and sodium-calcium exchanger (genes whose expression is increased during
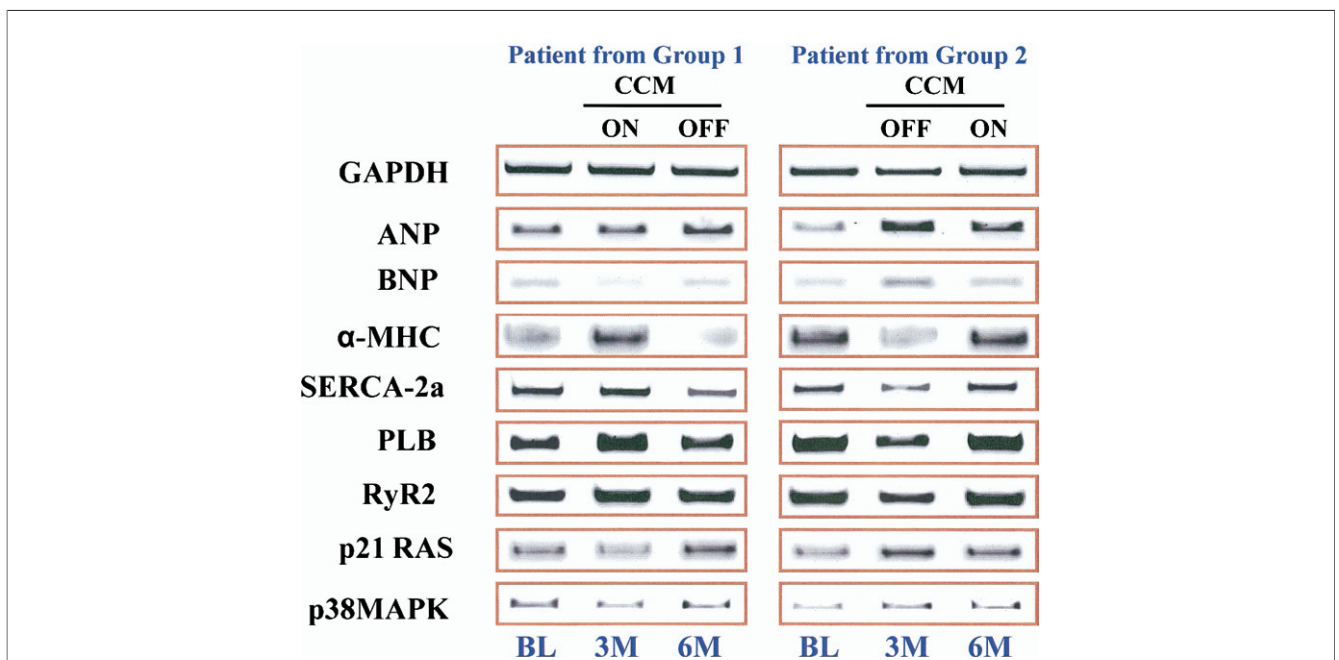

Figure 2   **Representative Northern Blots**

Representative Northern blots from 2 patients, 1 from each treatment group. Differences in band intensities between periods of active and sham treatment are evident. The GAPDH band intensities show consistency of ribonucleic acid loading of the various lanes. ANP = A-type natriuretic peptides; BL = baseline; BNP = B-type natriuretic peptides; CCM = cardiac contractility modulation; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; MHC = myosin heavy chain; PLB = phospholamban; p21 RAS = p21 Ras stretch receptor gene; p38MAPK = p38 mitogen activated protein kinase stretch receptor gene; RyR2 = ryanodine receptor; SERCA-2a = sarcoplasmic reticulum calcium adenosine triphosphatase.
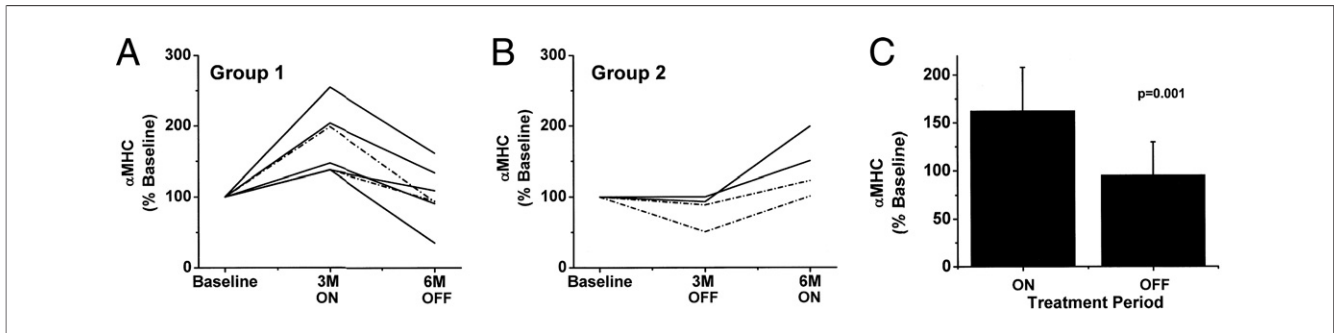
Figure 3   *α*-MHC Gene Expression

Changes in *α*-myosin heavy chain (MHC) gene expression (quantified as percent of baseline) at the end of study periods in group 1 **(A)** and group 2 **(B)** patients. **(C)** Data summarized by pooling of the end of ON periods from the 2 groups and end of OFF periods from the 2 groups. **Solid lines** show data from patients with idiopathic cardiomyopathy, whereas **dashed lines** are from patients with ischemic cardiomyopathy.

chronic HF) all decreased in response to 3 months of CCM signal treatment.

Clinical-molecular correlations. We explored correlations between changes in gene expression and clinical effects. Specifically, we correlated percent changes in expression of SERCA-2a and percent changes in peak V_{O_2}, MLHFS, and 6MW. These calculations were made by comparing 3-month data to baseline and also comparing 6- to 3-month data (Fig. 5). Despite the small number of patients, there were statistically significant correlations between changes in peak V_{O_2} and MLHFQ and the change in SERCA-2a expression. The correlation between SERCA-2a and 6MW

was not significant. These findings were similar for ischemic and idiopathic cardiomyopathy.

Discussion

We show that 3 months of CCM signal treatment is associated with improved expression of genes that are either abnormally down- or up-regulated in HF. No differences were identified in these responses in ischemic and idiopathic cardiomyopathies. Because no tissue samples were available from normal controls, all changes are reported relative to the baseline HF state. Therefore, it was not evaluated whether
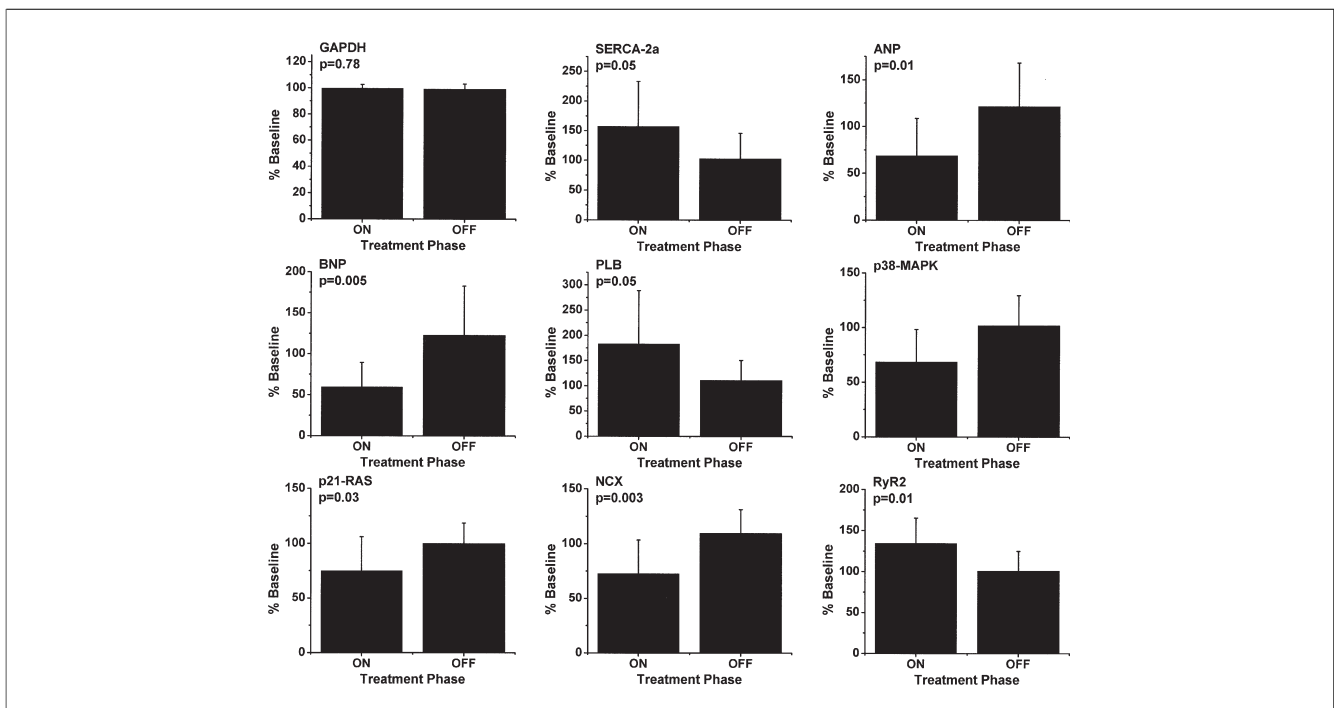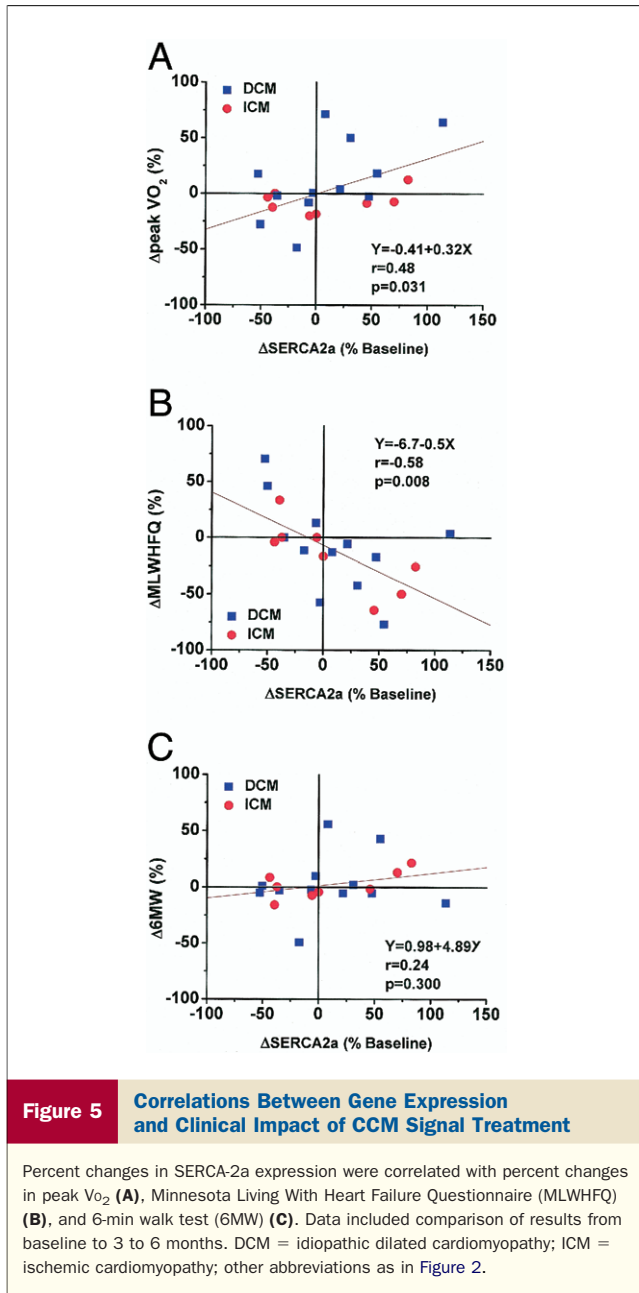

Figure 4   Summary of Impact of CCM Signal Treatment on Gene Expression

Summary of impact of cardiac contractility modulation (CCM) signal treatment on expression of genes examined in this study. Data for each gene analyzed as summarized in text and in the legend of Figure 3. NCX = sodium-calcium exchanger; other abbreviations as in Figure 2.

Figure 5   Correlations Between Gene Expression and Clinical Impact of CCM Signal Treatment

Percent changes in SERCA-2a expression were correlated with percent changes in peak Vo$_2$ **(A)**, Minnesota Living With Heart Failure Questionnaire (MLWHFQ) **(B)**, and 6-min walk test (6MW) **(C)**. Data included comparison of results from baseline to 3 to 6 months. DCM = idiopathic dilated cardiomyopathy; ICM = ischemic cardiomyopathy; other abbreviations as in Figure 2.

gene expression was normalized by CCM signal treatment, just that it improved toward the normal state. These findings are complementary to recently reported (8) effects of chronic CCM signal treatment in experimentally induced HF in dogs. It is noteworthy that, as in the prior experimental study, the clinical and molecular effects are identified in patients already taking chronic beta-blocker therapy.

Early studies of the mechanisms underlying the acute effects of CCM signals focused on their impact on action potential (5), peak intracellular calcium (2,14), and calcium loading of the sarcoplasmic reticulum. The increase in calcium was assumed to be the mechanism of increased contractility. More recent studies have suggested that CCM signals can have an impact on gene expression and phos-

phorylation of key proteins (e.g., phospholamban) involved with calcium cycling and other basic aspects of cell function within hours of signal application (8,15). The results of the present study extend these prior findings by showing they are relevant to diseased human myocardium. These findings are interesting in light of prior basic research showing that low frequency, low intensity electromagnetic fields can induce gene expression and modify enzymatic reactions (such as protein phosphorylation) within minutes in in vitro systems (16). It has been suggested that electromagnetic fields accelerate electron transfer reactions that could in turn stimulate transcription by interacting with electrons in DNA to destabilize the hydrogen bonds holding the 2 DNA strands together.

However, rather than invoking a direct effect of CCM signal treatment on gene expression, it is also possible that the changes in gene expression are secondary to alleviation of mechanical stresses brought about by local and global changes in myocardial function. For example, decreases in A- and B-type natriuretic peptides, p21 Ras, and p38 mitogen activated protein kinase are typically interpreted as indicating reduced mechanical stresses. Thus, alleviating excessive myocardial stretch may be responsible for down-regulation of stretch response proteins and block an important signaling pathway for HF progression. Nevertheless, it must be acknowledged that the mechanisms of action and the link between changes in gene expression and clinical effects are not clarified.

Study limitations. The main limitation of the present study is the small number of patients, which is due to the invasive nature of the techniques required. Nevertheless, the correlations between changes in myocardial gene expression and functional status indexed by either MLHFQ or peak Vo$_2$ were of great interest. Although these findings are provocative, it would be premature to suggest any mechanistic link given the limited data available at this time. The present study was also limited to an evaluation of tissue sampled from the right ventricular septum in the region where CCM signals are applied, leaving open the question if the results would extend to the left ventricle. In the prior animal study (8), CCM-mediated changes in gene expression were present in the region of signal application for the first several hours and present globally within 3 months. Because left heart biopsy was not possible, we cannot confirm that the changes identified would also be present in the remote areas of the myocardium. Also, because of the relatively small amount of tissue available from an endomyocardial biopsy, analysis of protein content and function were not possible. In our prior animal study (17), where abundant amounts of tissue were available, messenger RNA expression, protein content, and protein function were all assessed.

Conclusions

Application of CCM signals to the failing heart for 3 months is associated with improved expression of genes

associated with excitation-contraction coupling, and decreased expression of genes is associated with myocardial stress. These findings are present in tissue from patients with HF of either ischemic or nonischemic etiology. Both of these findings are indicative of beneficial molecular reverse remodeling. These findings are complementary to recent findings of the impact of CCM signals on gene expression in experimental HF (8). These findings can be extended to failing human myocardium. However, the mechanisms by which CCM signal treatment has an impact on gene expression are not known. It is clear that if CCM signal treatment improves myocardial stress on local and global levels, secondary normalization of gene expression can occur. As discussed previously (17), however, direct (primary) effects cannot be excluded. A randomized clinical trial, currently underway in the U.S., is designed to definitively test the safety and efficacy of this form of novel electrical therapy for chronic advanced HF.

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