

**Therapy With Cardiac Contractility Modulation Electrical Signals Improves
Left Ventricular Function and Remodeling in Dogs With Chronic Heart
Failure**

Makoto Imai, Sharad Rastogi, Ramesh C. Gupta, Sudhish Mishra, Victor G. Sharov,
William C. Stanley, Yuval Mika, Benny Rousso, Daniel Burkhoff, Shlomo
Ben-Haim, and Hani N. Sabbah

J. Am. Coll. Cardiol. 2007;49;2120-2128; originally published online May 11, 2007;

doi:10.1016/j.jacc.2006.10.082

This information is current as of November 21, 2010

The online version of this article, along with updated information and services, is
located on the World Wide Web at:

<http://content.onlinejacc.org/cgi/content/full/49/21/2120>

JACC

JOURNAL of the AMERICAN COLLEGE of CARDIOLOGY



PRECLINICAL STUDY

Therapy With Cardiac Contractility Modulation Electrical Signals Improves Left Ventricular Function and Remodeling in Dogs With Chronic Heart Failure

Makoto Imai, MD,* Sharad Rastogi, MD,* Ramesh C. Gupta, PHD,* Sudhish Mishra, PHD,* Victor G. Sharov, MD, PHD,* William C. Stanley, PHD,† Yuval Mika, PHD,‡ Benny Rousso, PHD,‡ Daniel Burkhoff, MD, PHD, FACC,‡ Shlomo Ben-Haim, MD, PHD,‡ Hani N. Sabbah, PHD, FACC*
Detroit, Michigan; Cleveland, Ohio; and Orangeburg, New York

- Objectives** This study examined the effects of long-term delivery of cardiac contractility modulation (CCM) electric signals on left ventricular (LV) function and global, cellular, and molecular remodeling in dogs with chronic heart failure (HF).
- Background** Acute studies in dogs with experimentally induced HF showed that CCM signals applied to the failing myocardium during the absolute refractory period improved LV function without increasing myocardial oxygen consumption.
- Methods** In one study, dogs with intracoronary microembolization-induced HF were randomized to 3 months of active CCM monotherapy or to a sham-operated control group. In another study, 19 HF dogs were randomized to 3 months chronic monotherapy with extended release metoprolol succinate (MET-ER), MET-ER with CCM, or no therapy at all (control group).
- Results** In CCM-only treated dogs, LV ejection fraction (EF) increased ($27 \pm 1\%$ vs. $33 \pm 1\%$, $p < 0.0001$) compared with a decrease in sham-operated control animals ($27 \pm 1\%$ vs. $23 \pm 1\%$, $p < 0.001$). The increase in EF seen with CCM-treated dogs was accompanied by reduced LV volumes, improved myocardial structure, reversal of the maladaptive fetal gene program, and an improvement in sarcoplasmic reticulum calcium cycling proteins. Dogs treated with a combination of MET-ER and CCM showed a greater increase in LV EF and a greater reversal of LV global, structural, and biochemical remodeling compared with dogs treated with MET-ER alone.
- Conclusions** In dogs with HF, long-term CCM therapy improves LV systolic function. The improvements are additive to those seen with beta-blockers. These findings are further strengthened by the concomitant benefits of CCM therapy on LV global, cellular, and biochemical remodeling. (J Am Coll Cardiol 2007;49:2120–8) © 2007 by the American College of Cardiology Foundation

Despite improvements in pharmacologic therapy for chronic heart failure (HF) (1–3), a large number of patients with New York Heart Association functional class III and IV are refractory to optimal standard medical therapy. The need for further therapeutic interventions in this patient population has given rise to a host of device-based therapies such as cardiac resynchronization therapy. Cardiac resynchronization therapy has been shown to improve left ventricular

(LV) systolic function in a subset of patients with dyssynchrony of myocardial contraction (4–7). Cardiac contractility modulation (CCM) electrical signals delivered to the failing myocardium during the absolute refractory period is another device-based therapy targeting this advanced HF population (8). In dogs with chronic HF, CCM signals applied acutely via leads sutured directly to the LV epicardium (9) or longer term via leads placed retrograde into the coronary sinus and positioned in the anterior coronary vein (10) resulted in improved LV systolic function (9,10). Preliminary clinical studies of CCM signals delivered to the myocardium of patients with chronic HF suggest that CCM therapy is safe and can also improve exercise tolerance and quality of life (11,12). In dogs with HF, classical positive inotropic agents such as dobutamine also improve LV systolic function but at a cost that the failing heart can ill afford, namely, an increase in myocardial oxygen con-

From the *Division of Cardiovascular Medicine, Henry Ford Heart and Vascular Institute, Detroit, Michigan; †Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio; and ‡Impulse Dynamics USA, Inc., Orangeburg, New York. The study was supported, in part, by a research grant from Impulse Dynamics USA, Inc. and by a grant from the National Heart, Lung, and Blood Institute, PO1 HL074237-03. Dr. Sabbah has research grant support from and is a consultant to Impulse Dynamics USA, Inc. Drs. Mika, Rousso, Burkhoff, and Ben-Haim are full-time employees of Impulse Dynamics USA, Inc.

Manuscript received August 11, 2006; accepted October 1, 2006.

sumption (MVO_2) (13). In contrast, acute delivery (2 h) of CCM therapy in dogs with chronic HF was associated with improved LV function and with no increase in MVO_2 (8). The purpose of this study was to determine: 1) whether long-term CCM monotherapy improves LV systolic function when used alone and in combination with a beta-blocker; 2) whether CCM therapy elicits improvement in LV remodeling both globally and at the cellular and molecular levels; and 3) potential mechanisms that underlie the improvement in LV function seen with CCM therapy.

Methods

Animal model. The dog model of chronic HF used in the present study was previously described in detail (14–16). In this study, healthy mongrel dogs weighing between 20 kg and 30 kg underwent coronary microembolizations to produce HF. Microembolizations were performed during cardiac catheterization under general anesthesia and sterile conditions. Animals were induced with intravenous oxymorphone hydrochloride (0.22 mg/kg) and diazepam (0.17 mg/kg), and a plane of anesthesia was maintained with 1% to 2% isoflurane. The study was approved by Henry Ford Health System Institutional Animal Care and Use Committee.

Implantation of the CCM system. Two weeks after the target LV ejection fraction (EF) was reached, dogs were anesthetized, intubated, and ventilated with room air. The right external jugular vein was surgically exposed and used to position the CCM leads. Two standard active fixation leads were advanced into the right ventricle, positioned on

the anterior and posterior septal grooves, and used to sense ventricular activity and deliver CCM electrical signals. A third lead was positioned in the right atrium for p-wave sensing (Fig. 1). The leads were connected to a CCM signal generator (OPTIMIZER II, Impulse Dynamics USA, Inc., Orangeburg, New York) implanted in a subcutaneous pocket created on the right side of the neck. As observed in clinical studies to date (8), there was no induction of ventricular or atrial ectopic beats, atrial fibrillation, or intercostal stimulation. Diaphragmatic stimulation was also avoided by testing signal application during the implant and moving the leads if a problem was observed. Studies were initiated 2 weeks after CCM system implantation to allow leads to stabilize in place.

Chronic study protocol—monotherapy with CCM.

Fourteen dogs were embolized to achieve a target LV EF, determined angiographically, of <30%. Two weeks after device implantation, dogs underwent a pretreatment left and right heart catheterization and were then randomized to 3 months of active treatment group (n = 7) or to a sham-operated control group (n = 7). In the active group, CCM therapy was administered for 5 h/day based on a duty cycle of 1 h ON and 3 h and 48 min OFF for 3 months. During ON periods, CCM signals

were delivered on every beat after a delay from the detection of electrical activity at one of the right ventricular leads. As detailed previously (12), the signals consisted of 2 biphasic pulses having a total pulse width of 20.56 ms (10.28 ms/pulse) with an amplitude of ± 7.73 V. Control dogs did not receive any therapy. At the end of 3 months of therapy or follow-up, all hemodynamic measurements were repeated. Finally, while under general anesthesia, the chest was opened and the heart rapidly harvested and tissue prepared for histological and biochemical evaluation. Tissue from 6 normal dogs were obtained and prepared in the same manner for comparisons. All tissue was stored at -70°C until needed.

Abbreviations and Acronyms

ANP = atrial natriuretic peptide
AR = adrenergic receptor
BNP = brain natriuretic peptide
CCM = cardiac contractility modulation
CD = capillary density
CSQ = calsequestrin
EDV = left ventricular end-diastolic volume
EF = ejection fraction
ESV = end-systolic volume
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
HF = heart failure
LV = left ventricular
MCSA = myocyte cross-sectional area
MET-ER = metoprolol succinate-extended release
MHC = α -myosin heavy chain
MVO_2 = myocardial oxygen consumption
ODD = oxygen diffusion distance
PLB = phospholamban
P-PLB = phosphorylated phospholamban
RyR = ryanodine receptor
SERCA-2a = sarcoplasmic reticulum calcium ATPase
SR = sarcoplasmic reticulum
VFIF = volume fraction of interstitial fibrosis
VFRF = volume fraction of replacement fibrosis

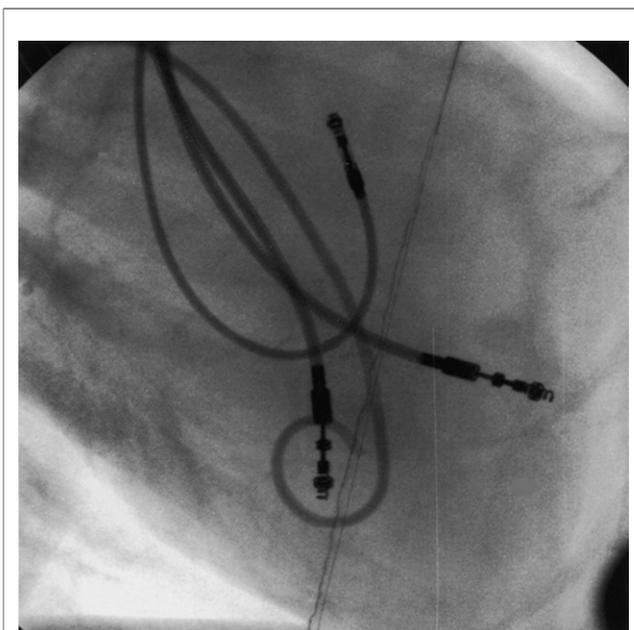


Figure 1 Right Anterior Oblique Fluoroscopic Image of Leads Placement During CCM System Implantation

CCM = cardiac contractility modulation.

Chronic study protocol—CCM in combination with beta-blockade. To determine whether CCM therapy has added benefits when used in combination with a beta-blocker, 19 dogs with HF (LV EF between 30% and 40%) were randomized to 3 months CCM therapy delivered as described above but in this case in combination with oral extended-release metoprolol succinate (MET-ER 100 mg once daily, $n = 7$), MET-ER alone (100 mg once daily, $n = 6$), or to no therapy at all (control group, $n = 6$). Hemodynamic and angiographic measurements were made at randomization and repeated at the end of 3 months, and LV tissue was obtained for analysis.

Additional acute protocol to assess mechanism of action. To partly address mechanisms of action of CCM therapy, 6 additional HF dogs were studied acutely with the chest open via a midsternotomy. Epicardial CCM leads were placed on the anterior wall between the 2nd and 3rd diagonal branches. Hemodynamic and angiographic measurements were made before and 2 h after continuous CCM signal delivery at 7.73 V. At 2 h, myocardial samples were obtained from the LV anterior wall in the region of the CCM leads and from the LV posterior wall remote from the CCM leads. Left ventricular tissue from 6 normal dogs and 6 HF dogs that were untreated was also obtained and used for comparisons.

Hemodynamic and ventriculographic measurements. Aortic and LV pressures were measured with catheter-tip micromanometers (Millar Instruments, Houston, Texas) during cardiac catheterization. Left ventricular end-diastolic pressure was measured from the LV waveform. Single-plane left ventriculograms were obtained as previously described (14–16). Left ventricular end-diastolic volume (EDV) and end-systolic volume (ESV) were calculated from ventricular silhouettes using the area-length method (17). Stroke volume was calculated as the difference between EDV and ESV. Ejection fraction was calculated as the difference between EDV and ESV divided by EDV times 100.

Protein expression. Calsequestrin (CSQ), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), ryanodine receptor (RyR), total phospholamban (PLB), phosphorylated PLB (P-PLB) at serine 16 and threonine 17, sarcoplasmic reticulum (SR) calcium ATPase (SERCA-2a), and β_1 -adrenergic receptor (AR) were measured in sodium dodecyl sulfate-LV homogenate prepared from LV powder by Western blots as described previously (18,19). Total P-PLB was quantified in sodium dodecyl sulfate-phosphoprotein-enriched fraction prepared from LV homogenate using PLB-specific monoclonal antibody (20). Band intensity was quantified using a Bio-Rad GS-670 imaging densitometer and expressed as densitometric units $\times \text{mm}^2$.

mRNA expression. The mRNA expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), α -myosin heavy chain (MHC), β_1 -AR, ANP, BNP, SERCA-2a, PLB, RyR, and CSQ was measured. Total RNA with an absorbance ratio (260 nm/280 nm) above 1.7 was isolated

from frozen LV tissue, and approximately 4 μg to 10 μg RNA was reverse-transcribed into cDNA in an assay volume of 80 μl as described previously (20). The mRNA expression of α -MHC was measured by amplification of cDNA by reverse transcriptase-polymerase chain reaction followed by digestion with Pst1 restriction enzyme as described previously (21). Fluorescent band intensity was quantified using a Bio-Rad GS-670 imaging densitometer and expressed as optical density $\times \text{mm}^2$.

Histomorphometric assessments. From each heart, 3 transverse slices (approximately 3-mm thick), 1 each from basal, middle, and apical thirds of the LV, were obtained. For comparison, tissue samples obtained from 7 normal dogs were prepared in an identical manner. From each slice, transmural tissue blocks were obtained and embedded in paraffin blocks. Transmural tissue blocks were also obtained from the free wall segment of the slice, mounted on cork using Tissue-Tek embedding medium (Sakura Finetek U.S.A. Inc., Torrance, California), and rapidly frozen in isopentane precooled in liquid nitrogen and stored at -70°C until used. The volume fraction of replacement fibrosis (VFRF), volume fraction of interstitial fibrosis (VFIF), myocyte cross-sectional area (MCSA), capillary density (CD), and oxygen diffusion distance (ODD) were measured as previously described (22–24).

Data analysis. To assess treatment effect, the change (Δ) in each measure from pretreatment (PRE) to post-treatment (POST) was calculated for each of the study groups and then the Δ compared between groups. For these comparisons, a t statistic for 2 means was used with a probability value ≤ 0.05 considered significant. Within-group comparisons between PRE and POST hemodynamic measures were made using a Student paired t test with a value of $p \leq 0.05$ considered significant. Between-group comparisons were examined using 1-way analysis of variance with α set at 0.05. If significance was achieved, pairwise comparisons were performed among groups using the Student-Newman-Kuels test with a probability value of ≤ 0.05 considered significant. All data are reported as the mean \pm SEM.

Results

Findings with chronic CCM monotherapy. At baseline, all dogs entered into the study had hemodynamic measures that were within normal limits for conditioned mongrel dogs in our laboratory. The hemodynamic and ventriculographic results obtained at PRE and POST are shown in Table 1. In sham-operated dogs, comparison of PRE to POST showed no differences in heart rate, systolic aortic pressure, LV end-diastolic pressure, or stroke volume. In this group, LV EDV and ESV increased significantly while LV EF decreased significantly (Table 1). In CCM-treated dogs, comparison of PRE to POST also showed no differences in heart rate and systolic aortic pressure. Left ventricular end-diastolic pressure decreased as did LV EDV and ESV while stroke volume and EF increased. Comparison of

Table 1 Hemodynamic and Angiographic Findings in Sham-Operated and CCM-Treated Dogs With Heart Failure Before and 3 Months After Initiating Treatment or Follow-Up

	Sham-Control Dogs			CCM-Treated Dogs		
	PRE	POST	p Value	PRE	POST	p Value
Heart rate (beats/min)	89 ± 4	101 ± 7	NS	78 ± 3	87 ± 3	NS
Systolic AoP (mm Hg)	97 ± 6	104 ± 6	NS	101 ± 4	102 ± 5	NS
LV EDP (mm Hg)	13 ± 1	14 ± 1	NS	14 ± 1	8 ± 1	0.01
LV EDV (ml)	67 ± 2	77 ± 2	0.0001	70 ± 6	66 ± 5	0.08
LV ESV (ml)	49 ± 2	60 ± 2	0.0001	52 ± 6	45 ± 4	0.008
LV EF (%)	27 ± 1	23 ± 1	0.001	27 ± 1	33 ± 1	0.0001
Stroke volume (ml)	18 ± 1	17 ± 1	NS	18 ± 1	21 ± 1	0.0001

AoP = aortic pressure; CCM = cardiac contractility modulation; EDP = end-diastolic pressure; EDV = end-diastolic volume; EF = ejection fraction; ESV = end-systolic volume; LV = left ventricular; NS = not significant; PRE = pretreatment; POST = post-treatment.

Δ (treatment effect) between the 2 study groups showed no change of heart rate and systolic aortic pressure. Compared with sham-operated control animals, CCM-treated dogs had a significantly lower LV end-diastolic pressure, EDV, and ESV along with significantly higher LV EF and stroke volume (Table 2).

Histomorphometric results are shown in Table 3. Volume fraction of replacement fibrosis, VFIF, and MCSA were significantly higher in sham-operated dogs compared with normal dogs. Volume fraction of replacement fibrosis was reduced by 23%, VFIF was reduced by 16%, and MCSA was reduced by 19% compared with sham-operated controls. Capillary density decreased in sham-operated controls while ODD increased when compared with normal dogs. Cardiac contractility modulation therapy restored CD and ODD to near normal (Table 3).

The mRNA expression in LV free wall of the housekeeping genes GAPDH and CSQ; the fetal program genes consisting of β₁-AR, αMHC, ANP, and BNP; and the cardiac sarcoplasmic reticulum (SR) genes SERCA-2a, PLB, and RyR are shown in Table 4. Expression of GAPDH and CSQ was unchanged among the 3 study groups, namely, normal dogs, sham-operated HF dogs, and HF CCM-treated dogs. mRNA expression of β₁-AR, αMHC, SERCA-2a, PLB, and RyR decreased, and expression of ANP and BNP increased significantly in sham-

operated HF dogs compared with normal animals. Cardiac contractility modulation therapy restored the expression of all genes to near normal levels. Protein expression in the LV free wall is shown in Table 4. Protein levels of CSQ were unchanged among the 3 study groups. Protein levels of β₁-AR, SERCA-2a, PLB, and RyR decreased and that of ANP and BNP increased significantly in sham-operated control animals compared with normal animals. Cardiac contractility modulation therapy restored the expression of all measured proteins except PLB. The restoration genes and proteins after 3 months of CCM therapy were the same in LV tissue obtained from the interventricular septum, the site nearest to the CCM signal delivery leads, and the LV free wall, a site remote from the CCM leads (Fig. 2). Protein levels of P-PLB at serine-16 and threonine-17 made in tissue obtained from both the interventricular septum and the LV free wall were significantly lower in sham-operated HF dogs compared with normal dogs and returned to near normal levels after 3 months of CCM therapy (Fig. 3, Table 5). In both the interventricular septum and LV free wall, the ratio of P-PLB at serine-16 to total PLB and the ratio of P-PLB at threonine-17 were also significantly lower in sham-operated HF dogs compared with normal dogs (Table 5). Long-term CCM therapy resulted in a significant increase of both ratios in

Table 2 Treatment Effect*

	Sham-Operated Untreated HF Dogs	CCM-Treated HF Dogs	p Value
Δ Heart rate (beats/min)	12 ± 8	8 ± 4	NS
Δ Systolic aortic pressure (mm Hg)	6 ± 6	1 ± 7	NS
Δ LV EDP (mm Hg)	1 ± 2	-6 ± 2	0.029
Δ LV EDV (ml)	10 ± 1	-4 ± 2	0.0001
Δ LV ESV (ml)	11 ± 1	-7 ± 2	0.0001
Δ LV EF (%)	-4 ± 1	6 ± 1	0.0001
Δ Stroke volume (ml)	-1 ± 0.5	3 ± 0.4	0.0001

*Comparison of the change (Δ) from pretreatment to post-treatment between sham-operated untreated heart failure (HF) dogs and CCM-treated HF dogs. p values based on comparison between sham-operated and CCM-treated dogs. Abbreviations as in Table 1.

Table 3 Chronic Histomorphometric Findings in Left Ventricular Myocardium of Normal Dogs, Sham-Operated Untreated HF Dogs, and CCM-Treated HF Dogs

	Normal Dogs	Sham-Operated Untreated HF Dogs	CCM-Treated HF Dogs
VFRF (%)	0.0	14.3 ± 1.5*	11.0 ± 0.8*†
VFIF (%)	3.7 ± 0.1	11.2 ± 0.3*	9.4 ± 0.7*†
MCSA (μm ²)	409 ± 10	719 ± 36*	581 ± 28*†
CD (# capillaries/mm ²)	2,607 ± 80	1,882 ± 67*	2,192 ± 117*†
CD (# capillaries/fiber)	1.00 ± 0.0	0.92 ± 0.02*	1.03 ± 0.02*†
ODD (μm)	8.9 ± 0.2	11.7 ± 0.3*	10.2 ± 0.2*†

*p < 0.05 versus normal dogs; †p < 0.05 versus sham-operated dogs.

CCM = cardiac contractility modulation electrical signals; CD = capillary density; HF = heart failure; MCSA = cardiomyocyte cross-sectional area; ODD = oxygen diffusion distance; VFIF = volume fraction of interstitial fibrosis; VFRF = volume fraction of replacement fibrosis.

Table 4 mRNA and Protein Expression of Fetal Program and Sarcoplasmic Reticulum Genes/Proteins in Left Ventricular Free Wall

	mRNA Expression (du)			Protein Expression (du)		
	NL	Sham	CCM	NL	Sham	CCM
β_1 -AR	22 ± 1	11 ± 1*	18 ± 1*†	102 ± 9	62 ± 10*	89 ± 7†
α -MHC	212 ± 7	148 ± 10*	180 ± 5*†	—	—	—
ANP	18 ± 2	34 ± 2*	27 ± 1*†	88 ± 7	147 ± 23*	63 ± 6†
BNP	23 ± 3	177 ± 4*	44 ± 5*†	65 ± 10	91 ± 4*	73 ± 3†
SERCA-2a	236 ± 4	154 ± 4*	192 ± 3*†	88 ± 7	54 ± 4*	73 ± 8†
PLB	232 ± 8	149 ± 8*	212 ± 7†	446 ± 19	277 ± 12*	299 ± 9*
RyR	38 ± 2	22 ± 2*	36 ± 5†	126 ± 8	88 ± 5*	106 ± 9†
CSQ	264 ± 5	275 ± 5	259 ± 14	43 ± 3	40 ± 1	44 ± 3
GAPDH	219 ± 6	223 ± 8	236 ± 4	—	—	—

*p < 0.05 versus normal dogs (NL); †p < 0.05 versus sham.
ANP = A-type natriuretic peptide; AR = adrenergic receptor; BNP = B-type natriuretic peptide; CCM = cardiac contractility modulation electrical signals; CSQ = calsequestrin; du = densitometric units; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; MHC = myosin heavy chain; PLB = phospholamban; RyR = ryanodine receptor; SERCA-2a = sarcoplasmic reticulum calcium ATPase.

both the interventricular septum and the LV free wall (Table 5).

Chronic CCM therapy in combination with beta-blockade. Ventriculographic results obtained at PRE and POST are shown in Table 6. Heart failure control animals POST showed a significant increase in LV EDV and ESV and a decrease in EF. Dogs treated with MET-ER alone showed a small reduction in LV EDV and a significant decrease in LV ESV while EF increased. Directionally similar results were seen with combination therapy of MET-ER and CCM (Table 6). Treatment effect analysis showed a significantly greater improvement of LV ESV and EF in dogs treated with a combination of MET-ER and CCM compared with dogs treated with MET-ER alone (Table 6).

Histomorphometric results are shown in Table 7. Volume fraction of replacement fibrosis, VFIF, MCSA, and ODD were significantly higher in HF control animals compared with normal dogs while CD was lower. Monotherapy with MET-ER reduced, albeit in part, VFRF, VFIF, MCSA, and ODD and increased CD (Table 7).

Combination therapy elicited a significantly greater improvement in VFIF, MCSA, CD, and ODD compared with MET-ER alone (Table 7).

Expression of SR proteins in the LV free wall is shown in Table 7. Compared with normal dogs, HF control animals showed decreased expression of SERCA-2a, RYR, P-PLB at serine-16, and P-PLB at threonine-17 with no change in CSQ or total PLB. Monotherapy with MET-ER significantly increased the expression of SERCA-2a, RYR, and P-PLB at serine-16 and threonine-17 again without a change in CSQ or total PLB. Combination therapy significantly increased the expression of P-PLB at serine-16 and threonine-17 above that seen with MET-ER alone (Fig. 4) but did not increase further the expression of SERCA-2a and RYR (Table 7).

Acute delivery of CCM therapy. Compared with baseline, continuous delivery of CCM therapy for 2 h increased LV EF (31 ± 2% vs. 26 ± 1%, p < 0.05). Compared with untreated HF dogs, the ratio of P-PLB to total PLB increased significantly in CCM-treated HF dogs compared with untreated HF dogs in the LV anterior wall at the site

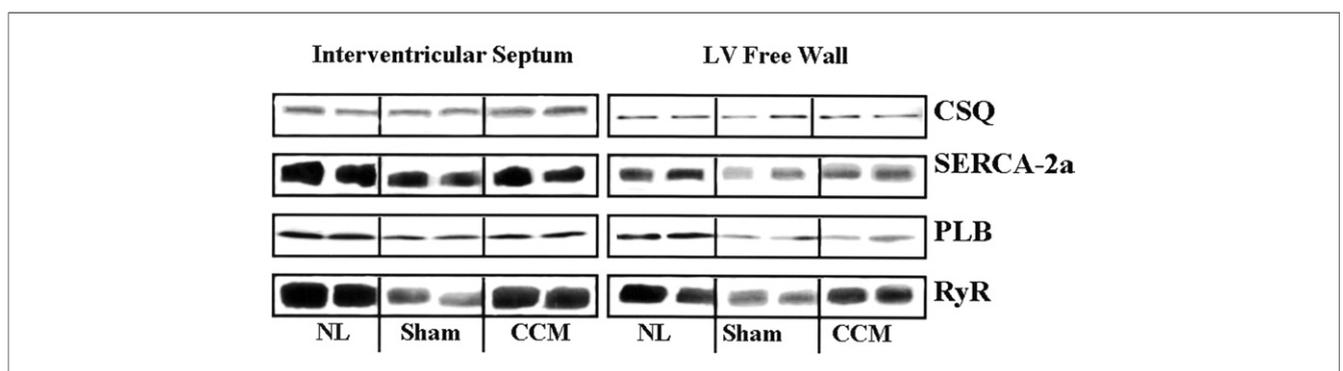
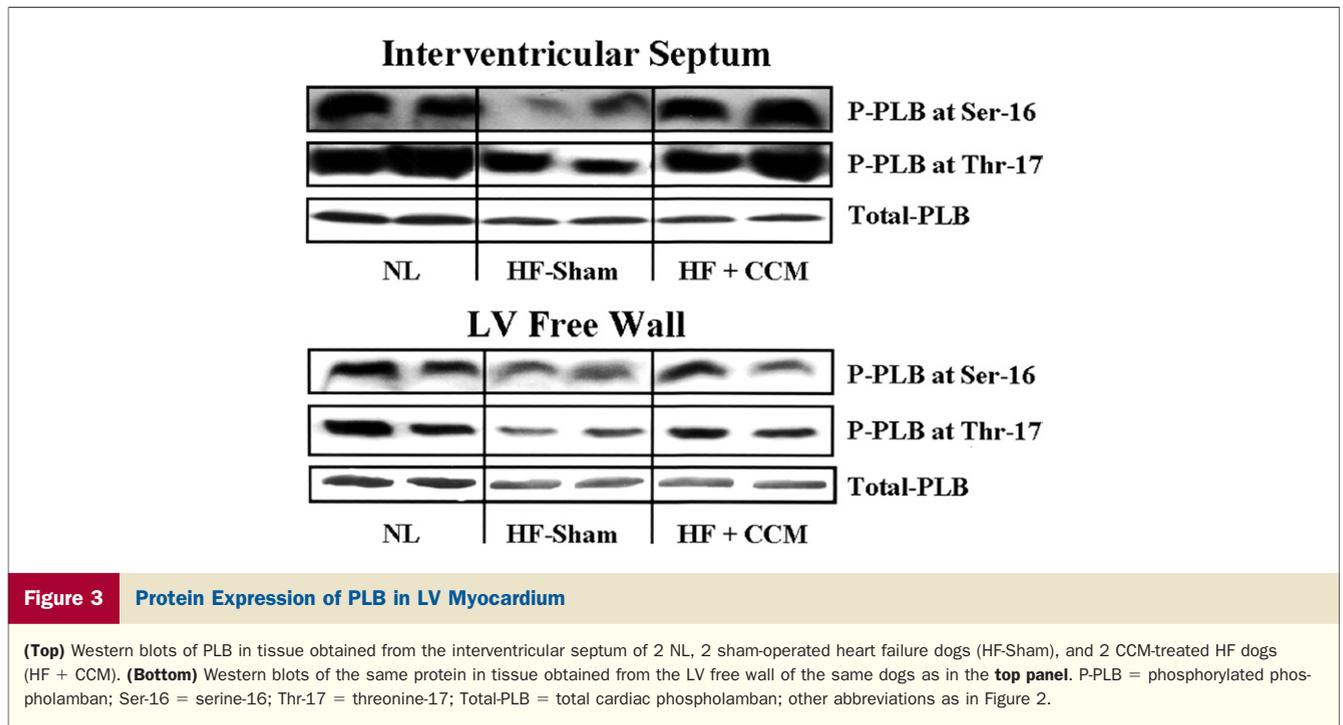


Figure 2 Expression of Sarcoplasmic Reticulum Proteins in LV Myocardium

(Left) Western blots of sarcoplasmic reticulum proteins in tissue obtained from the interventricular septum of 2 normal dogs (NL), 2 sham-operated heart failure dogs (Sham), and 2 cardiac contractility modulation (CCM)-treated dogs. (Right) Western blots of the same proteins in tissue obtained from the left ventricular (LV) free wall of the same dogs as in the left panel. CSQ = calsequestrin; PLB = phospholamban; RyR = ryanodine receptor; SERCA-2a = sarcoplasmic reticulum calcium ATPase.



of signal delivery (Fig. 5), whereas it was essentially unchanged in the LV posterior wall remote from the site of CCM signal delivery (Fig. 6).

Discussion

Results of this study indicate that in dogs with HF, long-term therapy with CCM electrical signals improves global LV function and attenuates global LV remodeling. Cardiac contractility modulation therapy also reverses many of the structural, biochemical, and molecular changes seen in untreated HF dogs. Results of the study also indicate that CCM therapy provides additive improvement when used on top of beta-blockade. Dogs treated with a combination of MET-ER and CCM showed a greater increase in LV EF and a greater reversal of LV global, structural, and biochemical remodeling compared with dogs treated with MET-ER alone.

Mechanisms of action of CCM therapy. Early studies of the mechanisms of action of CCM therapy were limited to acute signal application and focused on the potential impact

of CCM signals on action potential configuration, which only secondarily acts to enhance calcium loading of the SR (25,26). The increase in calcium was assumed to be the sole cause of the increase in contractility. Furthermore, the acute impact of CCM signals on contractile strength was shown to be limited to only the region of signal application. Thus, the results of the present studies significantly extend understanding of the effects of CCM signals in several ways. First, in the acute setting, CCM signals induce changes in gene expression. Indeed, significant prior research (27) has shown in in-vitro model systems that low-frequency, low-intensity electromagnetic fields can within minutes induce gene expression. It has been suggested that electromagnetic fields accelerate electron transfer reactions, which could, in turn, stimulate transcription by interacting with electrons in DNA to destabilize the hydrogen bonds holding the 2 DNA strands together. Evidence also suggests that electromagnetic fields are more effective in stimulating transcription when the fields are applied repeatedly at frequencies that coincide with the natural rhythms of the processes

Table 5 Protein Expression of Total PLB and P-PLB at Ser-16 and Thr-17 in the Interventricular Septum and LV Free Wall

	Interventricular Septum			LV Free Wall		
	NL	Sham	CCM	NL	Sham	CCM
Total PLB (du)	445 ± 7	305 ± 23*	299 ± 16*	446 ± 19	305 ± 19*	299 ± 9*
P-PLB Ser-16 (du)	85 ± 6	47 ± 5*	79 ± 6†	128 ± 11	50 ± 12*	87 ± 11*†
P-PLB Thr-17 (du)	146 ± 6	62 ± 10*	129 ± 12†	137 ± 4	56 ± 7*	109 ± 18†
P-PLB Ser-16/total PLB	0.19 ± 0.01	0.15 ± 0.01*	0.27 ± 0.02†	0.29 ± 0.03	0.16 ± 0.03*	0.29 ± 0.04†
P-PLB Thr-17/total PLB	0.33 ± 0.01	0.21 ± 0.04*	0.44 ± 0.05†	0.31 ± 0.02	0.19 ± 0.03*	0.36 ± 0.05†

*p < 0.05 versus NL; †p < 0.05 versus sham.

CCM = cardiac contractility modulation electrical signals; P-PLB = phosphorylated phospholamban; Ser-16 = serine-16; Thr-17 = threonine-17; other abbreviations as in Tables 1 and 4.

Table 6 Angiographic Findings

	HF Control Animals		HF + BB		HF + BB + CCM	
	PRE	POST	PRE	POST	PRE	POST
LV EDV (ml)	60 ± 1	64 ± 1*	64 ± 4	62 ± 5	70 ± 4	69 ± 4
LV ESV (ml)	39 ± 1	45 ± 1*	41 ± 2	36 ± 4*	45 ± 3	36 ± 3*
LV EF (%)	36 ± 1	31 ± 1*	36 ± 1	42 ± 2*	36 ± 1	48 ± 1*
Treatment effect						
Δ LV EDV (ml)		3.7 ± 0.8		-1.3 ± 1.0†		-0.9 ± 1†
Δ LV ESV (ml)		5.8 ± 0.8		-4.3 ± 1.0†		-7.9 ± 0.6†‡
Δ LV EF (%)		-5.3 ± 0.7		6.2 ± 1.0†		11.1 ± 0.6†‡

*p < 0.05 PRE versus POST; †p < 0.05 versus heart failure (HF) control animals; ‡p < 0.05 versus HF + BB.
BB = beta-blocker; other abbreviations as in Table 1.

affected (27). It is notable, therefore, that CCM signals are applied repeatedly (several hours per day) and in synchrony with the native heart beat. Changes in gene expression are unlikely to have acute functional effects since protein synthesis takes considerably longer time to occur. However, basic research has also shown that electromagnetic fields can also modify enzyme reactions. Interestingly, another acute effect identified in the present study is enhanced phosphorylation of PLB within as little as 2 h of signal application. In this case, the already existing PLB protein is being phosphorylated rather than synthesis of new protein. Phosphorylation of PLB enhances SR calcium sequestration by enhancing the activity and/or affinity of SERCA-2a for calcium. In turn, this enhances intracellular calcium cycling capacity and, hence, contractility. Finally, changes in intracellular calcium have themselves been linked to alterations in gene expression (28). Thus, there are at least 3 synergistic mechanisms by which CCM signals could immediately impact functional properties of cardiomyocytes in the region of signal application, which could mediate functional changes in the short and long term.

Effects of CCM therapy on LV remodeling. During chronic CCM therapy, normalization of gene expression may be a primary mechanism of functional improvements that may overshadow the impact on improved SR calcium

cycling identified in the short term. After chronic CCM therapy, molecular effects identified locally after acute CCM signal application are present in remote myocardium. These are also associated with reversal of cellular as well as structural remodeling. Remote effects may also be mediated by at least 2 mechanisms. First, the heart is a syncytium in which cardiomyocytes are connected by gap junctions, meaning that the intracellular environment of cells in 1 region can be transmitted to remote regions. Thus, over longer periods of time, local changes in intracellular environment may propagate to remote regions. Second, it has been shown that the acute effects of CCM therapy impact enough myocardium so as to enhance global LV function (8–10). If sustained over long periods of time, enhanced LV chamber contractility results in an overall more favorable hemodynamic state and mechanical loading condition on all cardiomyocytes. These factors would work secondarily to promote reverse remodeling of the myocardium in all regions of the heart, not just the region where CCM signals are delivered.

CCM therapy and beta-blockade. Therapeutic HF strategies such as beta-adrenergic blockade and cardiac resynchronization are associated with improved LV systolic function without an increase in MVO₂ and are each associated with improved outcomes (6,29). The abnormal gene

Table 7 Histomorphometric Findings and Expression of Sarcoplasmic Reticulum Calcium Cycling Proteins in Left Ventricular Myocardium

	NL	HF Control Animals	HF + BB	HF + BB + CCM
VFRF (%)	0.0	12.9 ± 1.5*	10.1 ± 1.7*†	10.3 ± 1.1*†
VFIF (%)	3.7 ± 0.1	14.2 ± 1.0*	9.7 ± 0.4*†	7.8 ± 0.2*†‡
MCSA (μm ²)	409 ± 10	674 ± 26*	562 ± 5*†	506 ± 4*†‡
CD (# capillaries/mm ²)	2607 ± 80	1756 ± 76*	2216 ± 25*†	2373 ± 18*†
CD (# capillaries/fiber)	1.00 ± 0.0	0.87 ± 0.03*	1.07 ± 0.02*†	1.08 ± 1*†
ODD (μm)	8.9 ± 0.2	11.8 ± 0.3*	10.5 ± 0.2*†	10.4 ± 0.2*†‡
SERCA-2a (du)	352 ± 12	238 ± 10*	321 ± 13†	422 ± 35*†‡
Total PLB (du)	79 ± 3	72 ± 2	73 ± 3	77 ± 3
RyR (du)	62 ± 2	45 ± 2*	59 ± 1†	62 ± 3†
CSQ (du)	371 ± 15	358 ± 8	362 ± 17	383 ± 13
P-PLB Ser-16 (du)	97 ± 8	65 ± 2*	77 ± 1*†	91 ± 4*†‡
P-PLB Thr-17 (du)	127 ± 3	70 ± 2*	91 ± 2*†	110 ± 6*†‡

*p < 0.05 versus NL; †p < 0.05 versus heart failure (HF) control animals; ‡p < 0.05 versus HF + BB.
BB = beta-blocker; CCM = cardiac contractility modulation; other abbreviations as in Tables 3, 4, and 5.

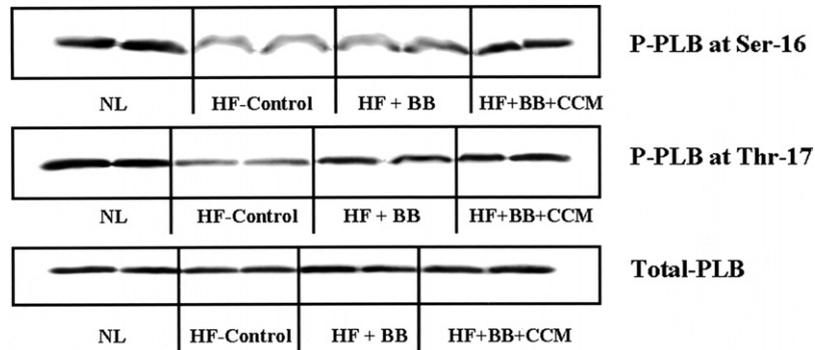


Figure 4 Protein Expression of PLB in LV Myocardium of Dogs Treated With a BB

Western blots of Total-PLB, P-PLB at Ser-16, and P-PLB at Thr-17 in tissue obtained from the LV free wall of 2 NL, 2 control untreated HF dogs (HF-Control), 2 HF dogs treated with beta-blockade only (HF + beta-blocker [BB]), and 2 HF dogs treated with both BB and CCM (HF + BB + CCM). Abbreviations as in Figures 2 and 3.

expression profile of HF, which recapitulates the fetal gene program, is reversed by beta-blocker treatment (30) as was also the case in the present study. As detailed in the preceding text, the impact of CCM treatment in this animal model of HF from a functional and remodeling perspective most closely mimics the impact of the beneficial effects seen with beta-blockers and cardiac resynchronization therapy. Results of the present study further indicate that CCM therapy can provide improvements in LV function and LV chamber remodeling that are additive to those seen with beta-blockade alone. This is particularly true with respect to SR calcium cycling protein and, in particular, PLB phosphorylation.

Study limitations. There are some study limitations that merit consideration. The study focused on identification of alteration in myocardial gene and protein expression during both acute (2 h) as well as chronic (3 months) delivery of CCM therapy. It remains uncertain, therefore, whether biochemical and molecular changes observed in myocardial regions remote from the site of CCM signal delivery required days, weeks, or months to manifest themselves. The CCM signal characteristics were chosen to be identical to those being used in ongoing clinical trials. Thus, it is not known if the observed effects can be enhanced if signal parameters such as amplitude, duration, and frequency are modified. The current study is limited to observation of

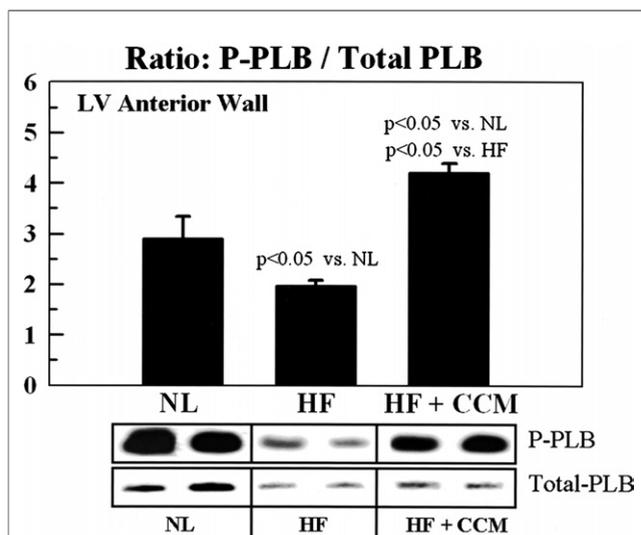


Figure 5 Ratio of P-PLB to Total PLB in the LV Anterior Wall

(Top) Bar graph (mean \pm SEM) depicting the ratio of P-PLB/Total PLB in LV anterior wall of 6 NL, 6 untreated HF dogs, and 6 CCM-treated HF dogs (HF + CCM). (Bottom) Western blots of P-PLB and Total-PLB in tissue obtained from the LV anterior wall of 2 NL, 2 untreated HF dogs, and 2 CCM-treated HF dogs (HF + CCM). Abbreviations as in Figures 2 and 3.

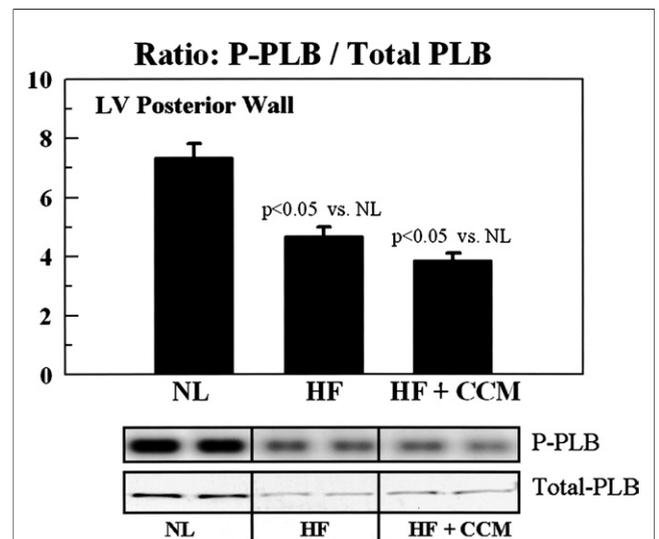


Figure 6 Ratio of P-PLB to Total-PLB in the LV Posterior Wall

(Top) Bar graph (mean \pm SEM) depicting the ratio of P-PLB/Total-PLB in LV posterior wall of 6 NL, 6 untreated HF dogs, and 6 CCM-treated HF dogs (HF + CCM). (Bottom) Western blots of P-PLB and Total-PLB in tissue obtained from the LV posterior wall of 2 NL, 2 untreated HF dogs, and 2 CCM-treated HF dogs (HF + CCM). Abbreviations as in Figures 2 and 3.

changes in expression and phosphorylation of only a small number of proteins that are abnormal in HF. The impact of CCM therapy on a host of other genes and proteins affected in HF is not addressed. While results of studies point to a possible role of PLB phosphorylation and perhaps reversal of the maladaptive fetal gene program as possible mechanisms of action of this form of therapy, additional studies are needed to demonstrate a cause and effect relationship.

Conclusions

Chronic CCM signal application in a clinically relevant animal model of HF is associated with marked improvement of global LV function and reversal of LV chamber remodeling both globally and at the cellular and molecular levels. The improvements are additive to those seen with beta-blockers. Cardiac contractility modulation therapy in this animal model of HF also reverses expression of genes known to be maladaptive in HF and was clearly associated with improved phosphorylation of PLB. The latter, along with improved expression of SERCA-2a, also a finding of this study, is integral to improvement of SR calcium cycling within cardiomyocytes and, hence, improved contractile performance. Confirmation of the clinical benefits of this investigational therapy, however, must await completion of ongoing clinical trials in patients with advanced HF.

Reprint requests and correspondence: Dr. Hani N. Sabbah, Cardiovascular Research, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, Michigan 48202. E-mail: HSABBAH1@hfhs.org.

REFERENCES

- Packer M, Coats AJ, Fowler MB, et al. Effect of carvedilol on survival in severe chronic heart failure. *N Engl J Med* 2001;344:1651–8.
- Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* 1999; 341:709–17.
- Moss AJ, Zareba W, Hall WJ, et al. Prophylactic implantation of a defibrillator in patients with myocardial infarction and reduced ejection fraction. *N Engl J Med* 2002;346:877–83.
- Cazeau S, Leclercq C, Lavergne T, et al. Effects of multisite biventricular pacing in patients with heart failure and intraventricular conduction delay. *N Engl J Med* 2001;344:873–80.
- Abraham WT, Fisher WG, Smith AL, et al. Cardiac resynchronization in chronic heart failure. *N Engl J Med* 2002;346:1845–53.
- Nelson GS, Berger RD, Fetics BJ, et al. Left ventricular or biventricular pacing improves cardiac function at diminished energy cost in patients with dilated cardiomyopathy and left bundle-branch block. *Circulation* 2000;102:3053–9.
- Auricchio A, Abraham WT. Cardiac resynchronization therapy: current state of the art: cost versus benefit. *Circulation* 2004;109: 300–7.
- Lawo T, Borggrefe M, Butter C, et al. Electrical signals applied during the absolute refractory period: an investigational treatment for advanced heart failure in patients with normal QRS duration. *J Am Coll Cardiol* 2005;46:2229–36.
- Morita H, Suzuki G, Haddad W, et al. Cardiac contractility modulation with nonexcitatory electric signals improves left ventricular function in dogs with chronic heart failure. *J Card Fail* 2003;9:69–75.
- Morita H, Suzuki G, Haddad W, et al. Long-term effects of non-excitatory cardiac contractility modulation electric signals on the progression of heart failure in dogs. *Eur J Heart Fail* 2004;6:145–50.
- Pappone C, Augello G, Rosanio S, et al. First human chronic experience with cardiac contractility modulation by nonexcitatory electrical currents for treating systolic heart failure: mid-term safety and efficacy results from a multicenter study. *J Cardiovasc Electro-physiol* 2004;15:418–27.
- Stix G, Borggrefe M, Wolpert C, et al. Chronic electrical stimulation during the absolute refractory period of the myocardium improves severe heart failure. *Eur Heart J* 2004;25:650–5.
- Chandler MP, Stanley WC, Morita H, et al. Acute treatment with ranolazine improves mechanical efficiency in dogs with chronic heart failure. *Circ Res* 2002;91:278–80.
- Sabbah HN, Stein PD, Kono T, et al. A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. *Am J Physiol* 1991;260:H1379–84.
- Sabbah HN, Shimoyama H, Kono T, et al. Effects of long-term monotherapy with enalapril, metoprolol and digoxin on the progression of left ventricular dysfunction and dilation in dogs with reduced ejection fraction. *Circulation* 1994;89:2852–9.
- Sabbah HN, Sharov VG, Gupta RC, et al. Reversal of chronic molecular and cellular abnormalities due to heart failure by passive mechanical ventricular containment. *Circ Res* 2003;93:1095–101.
- Dodge HT, Sandler H, Baxley WA, Hawley RR. Usefulness and limitations of radiographic methods for determining left ventricular volume. *Am J Cardiol* 1966;18:10–24.
- Gupta RC, Mishra S, Mishima T, Goldstein S, Sabbah HN. Reduced sarcoplasmic Reticulum Ca²⁺-uptake and PLB expression in ventricular myocardium of dogs with heart failure. *J Moll Cell Cardiol* 1999;31:1381–9.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1951;27:680–5.
- Gupta RC, Mishra S, Yang XP, Sabbah HN. Reduced inhibitor 1 and 2 activity is associated with increased protein phosphatase type 1 activity in left ventricular myocardium of one-kidney, one-clip hypertensive rats. *Mol Cell Biochem* 2005;269:49–57.
- Feldman AM, Ray PE, Silan CM, Mercer JA, Minobe W, Bristow MR. Selective gene expression in failing human heart: quantification of steady-state levels of messenger RNA in endomyocardial biopsies using the polymerase chain reaction. *Circulation* 1991;83:1866–72.
- Liu YH, Yang XP, Sharov VG, et al. Effects of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists in rats with heart failure. Role of kinins and angiotensin II type 2 receptors. *J Clin Invest* 1997;99:1926–35.
- Suzuki G, Morita H, Mishima T, et al. Effects of long-term monotherapy with eplerenone, a novel aldosterone receptor blocker, on the progression of left ventricular dysfunction and remodeling in dogs with heart failure. *Circulation* 2002;106:2967–71.
- Morita H, Suzuki G, Chaudhry PA, et al. Effects of long-term monotherapy with metoprolol CR/XL on the progression of left ventricular dysfunction and remodeling in dogs with chronic heart failure. *Cardiovasc Drugs Ther* 2002;16:433–49.
- Burkhoff D, Shemer I, Felzen B, et al. Electric currents applied during the refractory period can modulate cardiac contractility in vitro and in vivo. *Heart Fail Rev* 2001;6:27–34.
- Mohri S, He KL, Dickstein M, et al. Cardiac contractility modulation by electric currents applied during the refractory period. *Am J Physiol* 2002;282:H1642–7.
- Blank M, Goodman R. Initial interactions in electromagnetic field-induced biosynthesis. *J Cell Physiol* 2004;199:359–63.
- Marban E, Koretsune Y. Cell calcium, oncogenes, and hypertrophy. *Hypertension* 1990;15:652–8.
- The MERIT-HF Investigators. Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure (MERIT-HF). *Lancet* 1999;353:2001–7.
- Lowes BD, Gilbert EM, Abraham WT, et al. Myocardial gene expression in dilated cardiomyopathy treated with beta-blocking agents. *N Engl J Med* 2002;346:1357–65.

Therapy With Cardiac Contractility Modulation Electrical Signals Improves Left Ventricular Function and Remodeling in Dogs With Chronic Heart Failure

Makoto Imai, Sharad Rastogi, Ramesh C. Gupta, Sudhish Mishra, Victor G. Sharov, William C. Stanley, Yuval Mika, Benny Rousso, Daniel Burkhoff, Shlomo Ben-Haim, and Hani N. Sabbah

J. Am. Coll. Cardiol. 2007;49;2120-2128; originally published online May 11, 2007;

doi:10.1016/j.jacc.2006.10.082

This information is current as of November 21, 2010

Updated Information & Services	including high-resolution figures, can be found at: http://content.onlinejacc.org/cgi/content/full/49/21/2120
References	This article cites 30 articles, 11 of which you can access for free at: http://content.onlinejacc.org/cgi/content/full/49/21/2120#BIBL
Citations	This article has been cited by 9 HighWire-hosted articles: http://content.onlinejacc.org/cgi/content/full/49/21/2120#otherarticles
Rights & Permissions	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://content.onlinejacc.org/misc/permissions.dtl
Reprints	Information about ordering reprints can be found online: http://content.onlinejacc.org/misc/reprints.dtl