Myocardial Creatine Kinase Expression After Left Ventricular Assist Device Support

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Objectives We examined whether unloading of the left ventricle with a ventricular assist device (LVAD) can result in normalization of the creatine kinase (CK) abnormalities in the failing human heart.

Background Left ventricular failure is associated with a decrease of myocardial total CK activity and a fetal shift in CK isoform expression that results in an increase in the cytosolic brain type homodimeric-creatine kinase (CK-B) subunit and decreases of the cytosolic muscle-creatine kinase (CK-M) and CK-mitochondrial (CK-Mt) isoforms. The mechanisms of this abnormality are not known.

Methods Total CK activity and CK protein isoform expression (Western blotting) were examined in 11 patients with end-stage cardiomyopathy. In 7 patients, myocardial tissue was also obtained after 4.1 ± 1.1 months of left ventricular assist device (LVAD) support.

Results Left ventricular unloading produced by LVAD implantation resulted in a 270% ± 114% increase in total CK activity (p < 0.01) that was associated with a 69% ± 18% increase in CK-Mt protein expression (p < 0.01) and a 121% ± 69% increase in CK-Mt protein expression (p < 0.01), but no significant change in CK-B expression.

Conclusions Systolic and diastolic unloading provided by the LVAD resulted in increases of total CK activity as well as CK-Mt and CK-M protein expression. The failure of CK-B expression to decrease suggests that abnormalities other than increased loading are responsible for the increase in CK-B expression in the failing heart.

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In the heart adenosine triphosphate (ATP) is mainly synthesized in the mitochondria through oxidative phosphorylation and transported to the contractile apparatus, where it is consumed by myosin ATPase to generate force. In the normal heart, the creatine kinase (CK) system appears to facilitate ATP production, transport and utilization (1,2). Creatine kinase catalyzes the near-equilibrium reaction, transferring a phosphoryl group between ATP and creatine without loss of free energy: phosphocreatine (PCr) + magnesium (Mg) adenosine 5′-diphosphate (ADP) + H+ ⇌ creatine + Mg ATP. Creatine kinase consists of two subunits, cytosolic muscle-creatine kinase (CK-M) and cytosolic brain type homodimeric-creatine kinase (CK-B), resulting in three dimeric isoenzymes that are found in the cytosol: MM, MB and BB (3). The CK-MM isoenzyme is localized to the thick filaments of the contractile apparatus, where ADP generated by myosin ATPase during contraction can be rapidly rephosphorylated (4). CK-B is present mainly in fetal myocardium and is normally found only in trace amounts in the adult heart (5). A fourth isoform, CK-mitochondrial (CK-Mt), is found on the inner mitochondrial membrane in association with adenine nucleotide translocase; by transferring a phosphoryl group from ATP to creatine to form PCr, CK-Mt increases ADP availability to the ATP synthase (6). In failing hearts, a fetal shift in CK isoform expression occurs with an increase in the CK-B containing isoform and decreases in CK-MM (3,7–9), CK-Mt and total CK activity. It has been postulated that the fetal shift in CK enzyme expression is stimulated by a sustained increase in wall stress (3) and that it can be attenuated or reversed by pharmacologic unloading of the left ventricle (8,10,11).

Left ventricular assist devices (LVAD) are currently being used as bridge-to-transplant therapy in patients with severe heart failure (12,13). This provides the opportunity to determine whether the marked systolic and diastolic unloading of the dysfunctional left ventricle (LV) provided by this device can result in normalization of the CK abnormalities in the failing human heart. We observed that in response to unloading with the LVAD, total CK activity, CK-M and CK-Mt protein expression increased markedly. However, CK-B expression failed to decrease, suggesting that this abnormality is not merely the result of abnormally increased LV wall stress.

Materials and Methods

Patient selection. Eleven patients (mean age 50 years; range 27 to 61 years) with end-stage idiopathic/ischemic cardiomyopathy and no other end organ failure were studied. Mean LV ejection fraction was 18% before LVAD implantation. Of the 11 patients who had LVAD implantation, specimens were also available from seven of the...
The protein subunits were transferred to a nitrocellulose membrane and incubated with primary antibody (1:3,000 dilution in Tween-Tris-buffered saline; 0.1% Tween-20, 20 mM Tris base, 137 mM NaCl, pH 7.6) for 2 h. Monoclonal mouse antibodies specific to CK-M and CK-B (OEM Concepts Inc., Toms River, New Jersey) were directed against their respective protein subunits bound to the membrane. A rabbit polyclonal antibody specific for CK-Mt subunit (from A. W. Strauss, Washington University) was used at a dilution of 1:2,000. Membranes were washed and incubated with the appropriate secondary horseradish peroxidase-labeled (antimouse or antirabbit) immunoglobulin G antibody, incubated for 1 min with the chemiluminescent substrate (ECL, Amersham, Buckinghamshire, United Kingdom) and exposed to X-ray film for 15 s to 10 min. Membranes were stripped and sequentially reprobed. Densitometry was used for relative quantitation of the CK protein subunits normalized to beta-actin.

**Total CK and CK activity.** Total CK activity (IU/mg protein) was measured using a Cal Biochem CK-nac reagent kit (Sigma) (15). Homogenate activities in excess of 1,000 U/l were diluted so that total activity did not exceed the linearity of the system.

**Statistical analysis.** Data from the pre-LVAD and post-LVAD specimens were analyzed using the two-tailed unpaired t test and the paired t test, with the level of significance set at 0.05. All results are reported as mean ± SEM.

### RESULTS

**Patient characteristics.** The clinical characteristics of the 11 patients are summarized in Table 1. All patients were New York Heart Association functional class IV at LVAD implantation.

**Western blot analysis.** Representative Western blots of CK-M, CK-B and CK-Mt demonstrated migrations to positions corresponding to 40 to 45 kd on 12% SDS-polyacrylamide gel (Fig. 1). Densitometry of the protein levels normalized to beta-actin showed a mean CK-M subunit value of 1.18 ± 0.35 pre-LVAD (n = 11) and

### Table 1. Clinical Characteristics of Patients With End-Stage Dilated or Ischemic Cardiomyopathy

<table>
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<tr>
<th>Patient</th>
<th>Diagnosis</th>
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<th>EF (%)</th>
<th>CO (l/min)</th>
<th>HR (beats/min)</th>
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Mean ± SEM: 49 ± 2.7 EF 18 ± 1.9 CO 4.7 ± 0.48 HR 99 ± 3.6 SBP 96 ± 3.3 DBP 55 ± 3.5

CO = cardiac output; DBP = diastolic blood pressure (cuff); DCM = dilated cardiomyopathy; EF = ejection fraction; HR = heart rate; SBP = systolic blood pressure (cuff).
Figure 1. Densitometric intensities for protein bands from Western blots of creatine kinase (CK)-M, CK-B and creatine kinase-mitochondrial (CK-Mt) isoforms normalized to beta-actin. A through F represents six individual patients before and after left ventricular assist device (LVAD) implantation. The protein levels of CK-M and CK-Mt isoforms were increased after LVAD implantation while CK-B and beta-actin were unchanged.

Figure 2. Densitometric intensities for protein bands from Western blots of creatine kinase (CK)-M (A), CK-B (B) and creatine kinase-mitochondrial (CK-Mt) (C) isoforms normalized to beta-actin. The ratio of CK-M and CK-Mt to beta-actin increased significantly after hemodynamic unloading provided by the left ventricular assist device (LVAD). Values are mean ± SEM. *p < 0.05 versus pre-LVAD. Black bars = pre-LVAD; white bar = post-LVAD.
1.85 ± 0.17 post-LVAD (p < 0.01; n = 7) (Fig. 2). The normalized CK-Mt protein expression level was 1.31 ± 0.27 pre-LVAD (p < 0.05) and 2.01 ± 0.16 post-LVAD (p < 0.05). CK-B expression was not different pre-LVAD (2.02 ± 0.31) and post-LVAD (2.24 ± 0.30). In the paired analysis of seven patients from whom samples were available both pre- and post-LVAD implantation, CK-M subunit expression was increased by 59% (Fig. 3; 1.16 ± 0.15 vs. 1.85 ± 0.17, p < 0.01), CK-Mt protein expression was increased by 53% (Fig. 3; 1.31 ± 0.26 vs. 2.01 ± 0.16, p < 0.01), whereas CK-B subunit expression was not significantly changed.

**Myocardial total CK activity.** Chemically measured total CK activity normalized to cardiac protein for all 11 patients was 6.2 ± 1.13 IU/mg pre-LVAD and 14.37 ± 2.56 IU/mg post-LVAD (p < 0.01). In the paired analysis of the seven patients from whom myocardial specimens were available both pre-LVAD and post-LVAD, total CK activity increased by 185% following LVAD implantation (from 5.1 ± 1.42 to 14.4 ± 2.56 IU/mg protein, p < 0.01) (Fig. 3).

**DISCUSSION**

**CK activity in normal and failing hearts.** An altered expression of CK protein and activity is observed in failing hearts (16). Although normal myocardial tissue was not available in the present study, several investigators have previously reported CK activity and isoform distribution in normal human hearts. Nascimben et al. (7) reported total...
CK activity of 11.6 ± 2.4 IU/mg protein in surgical and autopsy specimens from patients without known cardiac disease. In that study CK activity was decreased to 7.7 ± 1.9 IU/mg protein in patients with dilated cardiomyopathy undergoing cardiac transplantation. This value is similar to the total CK activity of 6.2 ± 1.1 IU/mg protein in the patients undergoing LVAD implantation in the present study. Bristow et al. (17) reported that total CK activity was 22% lower in LV tissue obtained from hearts removed at cardiac transplantation than that from nonfailing myocardium obtained from brain-dead organ donors. Sylven et al. (18) found that CK activity in the LV of failing hearts was 44% lower than in control hearts. Interestingly, Nascimben et al. (7) reported that total CK activity was significantly lower in donor hearts than in myocardium obtained from accident victims, suggesting that subjects maintained on life support have a decrease of total CK activity. In the present study CK activity increased from 6.2 IU/mg in failing hearts to 14.4 IU/mg in the post-LVAD group, indicating that unloading of the left ventricle is associated with restoration of total CK activity toward normal.

CK isoform expression. Concomitant with the decrease in total CK activity, LV overload or failure is associated with a fetal shift in CK isoform expression (16). Thus, in patients with dilated cardiomyopathy, Nascimben (7) reported that CK-MB was increased from 2.4 ± 4% of total CK to 27 ± 6%; CK-MM was decreased from 88 ± 6% to 63% of total CK, while CK-Mt was unchanged at 9% to 10% of total CK. Similarly, animal models of LV pressure overload resulting from aortic stenosis or volume overload secondary

Figure 3. (Continued) CK-B normalized to beta-actin in patients without known cardiac disease. In that study CK activity was decreased to 7.7 ± 1.9 IU/mg protein in patients with dilated cardiomyopathy undergoing cardiac transplantation. This value is similar to the total CK activity of 6.2 ± 1.1 IU/mg protein in the patients undergoing LVAD implantation in the present study. Bristow et al. (17) reported that total CK activity was 22% lower in LV tissue obtained from hearts removed at cardiac transplantation than that from nonfailing myocardium obtained from brain-dead organ donors. Sylven et al. (18) found that CK activity in the LV of failing hearts was 44% lower than in control hearts. Interestingly, Nascimben et al. (7) reported that total CK activity was significantly lower in donor hearts than in myocardium obtained from accident victims, suggesting that subjects maintained on life support have a decrease of total CK activity. In the present study CK activity increased from 6.2 IU/mg in failing hearts to 14.4 IU/mg in the post-LVAD group, indicating that unloading of the left ventricle is associated with restoration of total CK activity toward normal.

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to mitral regurgitation demonstrate marked increases in CK-B protein expression (10, 19, 20). In comparison with myocardium from organ donors, papillary muscle specimens obtained from patients undergoing surgery for mitral regurgitation demonstrated no difference in total CK activity or CK-M activity, but CK-Mt was 40% lower and CK-B was 15- to 20-fold higher than in the normal hearts (18). These investigators suggested that a reciprocal relationship might exist between the CK-B and CK-Mt isoforms. However, in a study of mice lacking genes for either CK-M or CK-Mt, Saupe et al. (21) demonstrated that loss of either CK-M or CK-Mt had no effect on CK-B protein expression. These investigators also observed that the intrinsic activities of CK-BB and CK-MM isoenzymes were equivalent, suggesting that the isoenzyme shift toward the fetal pattern in the failing hearts confers no kinetic advantage (21).

**Effect of ventricular unloading.** Several investigators have examined CK isofrom distribution following pharmacologic vasodilator therapy in overloaded or failing hearts. Schultz et al. (20) reported that in dogs with chronic volume overload due to mitral regurgitation, treatment with the angiotensin-converting enzyme inhibitor ramipril resulted in normalization of left ventricular filling pressure, but did not decrease CK-B gene or protein expression. Similarly, Pauletto et al. (22) demonstrated that captopril treatment of rats with renovascular hypertension reduced blood pressure and normalized left ventricular mass, but did not reverse the increased expression of CK-MB. In contrast, in studies of pressure overload hypertrophy using the two-kidney-one-clip rat hypertension model, normalization of blood pressure with hydralazine did reverse the CK isoenzyme switch despite persistent hypertrophy (10). Left ventricular unloading is much greater with LVAD placement than with pharmacologic therapy. As with drug therapy, there is also a tendency to normalize neurohormonal and reflex activity following LVAD placement so that it is difficult to separate the direct effects of mechanical unloading from the neurohormonal alterations following LVAD placement. In the present study LVAD placement caused prominent increases of both CK-M and CK-Mt subunit expression as well as CK total activity. However, CK-B did not decrease. The reason for the persistently elevated CK-B expression is unclear. Nevertheless, from a functional point of view the prominent increases of CK-M and CK-Mt, as well as total CK activity, would be expected to restore CK kinetics toward normal. It is of interest that the 1.6-fold increase in CK-M and the 1.54-fold increase in CK-Mt protein expression were associated with a more than doubling of CK activity. The reason for this apparent discrepancy between protein expression and activity is uncertain, but may be in part related to the faster kinetics of CK-Mt.

Although the increased systolic wall stress of the dilated, failing left ventricle might be expected to increase energy consumption, several investigators have reported that coronary blood flow and myocardial oxygen consumption are decreased in the failing heart (23). In support of this finding, studies of isolated muscle strips from failing human hearts demonstrated reduced heat liberation by both tension-dependent (actin-myosin cross-bridging) and tension-independent (calcium cycling) mechanisms, suggesting downregulation of energy utilization processes (24). Other enzyme systems involved in energy production including the mitochondrial F$_{0}$F$_{1}$-ATPase are also decreased in the failing heart (25). These findings might suggest that the decreases of CK-M and CK-Mt are also a response to decreased energy-utilizing processes in the failing heart. If this were the case, however, then the marked reduction of systolic work resulting from LVAD implantation would have been expected to cause a further decrease of CK-M and CK-Mt as a result of the decrease in myocardial ATP demand. In fact, the opposite occurred. Unloading of the ventricle was associated with increases of both CK-M and CK-Mt to levels similar to those reported in normal hearts (7). These findings indicate that the decreases of CK-M and CK-Mt in the failing heart are not merely responses to a decrease of energy utilization processes, but likely represent a more fundamental alteration of protein expression in the failing heart.

**Functional implications of CK alterations.** A critical question is whether the abnormalities of the CK system have the potential to contribute to contractile dysfunction in the failing heart. The CK system is thought to optimize myocardial energy metabolism by maintaining ADP levels high at the mitochondria where ATP is generated and low at sites of ATP utilization, thereby contributing to maintenance of a high free energy for ATP hydrolysis to enhance the efficiency of contraction (1). Acutely inhibiting CK activity with iodoacetamide or replacing myocardial creatine by feeding with poorly hydrolyzable creatine analog impair the maximal cardiac workloads that can be achieved in response to catecholamine stimulation or increased perfusate calcium (26, 27). Hearts from mice lacking genes for either CK-M or both CK-M and CK-Mt perform normally during basal conditions but may have impairment of maximal contractile performance, and require increased cytosolic free ADP levels to achieve any given level of contractile work (21, 28). Nevertheless, even during severe reductions of CK activity, contractile performance appears to be impaired only at very high cardiac workloads. Furthermore, Roman et al. (29) have demonstrated in mouse skeletal muscle that there is functional equivalence of the CK isoforms, so that the alteration of isofrom expression in the patients with heart failure would not be expected to have a substantial effect on cardiac contractile performance. Because CK flux in the normal heart is at least an order of magnitude faster than the rate of ATP synthesis, the moderate reductions of total CK activity in the failing hearts would not be expected to result in myocardial dysfunction (30, 31). Thus, it is not clear whether the marked decrease of CK activity in the cardiomyopathic hearts contributed to the development of cardiac failure.

In conclusion, the systolic and diastolic unloading pro-
vided by the LVAD resulted in increases of CK-M and CK-Mt protein expression that were associated with increases of total CK activity. These changes would be expected to facilitate intracellular phosphoryl transfer from sites of energy production to sites of energy utilization, and to allow maintenance of lower cytosolic free ADP levels at any level of oxygen utilization. Whether these changes in response to mechanical unloading have the potential to improve contractile performance in the failing myocardium will require further study.

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REFERENCES