Relation Between Myocardial Function and Expression of Sarcoplasmic Reticulum Ca\(^{2+}\)-ATPase in Failing and Nonfailing Human Myocardium

Gerd Hasenfuss, Hans Reinecke, Roland Studer, Markus Meyer, Burkert Pieske, Jürgen Holtz, Christian Holubarsch, Herbert Posival, Hanjörg Just, Helmut Drexler

**Abstract** Expression of sarcoplasmic reticulum (SR) Ca\(^{2+}\)-ATPase was shown to be reduced in failing human myocardium. The functional relevance of this finding, however, is not known. We investigated the relation between myocardial function and protein levels of SR Ca\(^{2+}\)-ATPase in nonfailing human myocardium (8 muscle strips from 4 hearts) and in myocardium from end-stage failing hearts with dilated (10 muscle strips from 9 hearts) or ischemic (7 muscle strips from 5 hearts) cardiomyopathy. Myocardial function was evaluated by the force-frequency relation in isometrically contracting muscle strip preparations (37°C, 30 to 180 min\(^{-1}\)). In nonfailing myocardium, twitch tension rose with increasing rates of stimulation and was 76% higher at 120 min\(^{-1}\) compared with 30 min\(^{-1}\) (P<.02). In failing myocardium, there was no significant increase in average tension at stimulation rates above 30 min\(^{-1}\). At 120 min\(^{-1}\), twitch tension was decreased by 59% (P<.05) in dilated cardiomyopathy and 76% (P<.05) in ischemic cardiomyopathy compared with nonfailing myocardium. Protein levels of SR Ca\(^{2+}\)-ATPase, normalized per total protein or per myosin, were reduced by 36% (P<.02) or 32% (P<.05), respectively, in failing compared with nonfailing myocardium. SR Ca\(^{2+}\)-ATPase protein levels were closely related to SR Ca\(^{2+}\) uptake, measured in homogenates from the same hearts (r=.70, n=16, and P<.005). For all types of myocardium, there was a significant correlation between SR Ca\(^{2+}\)-ATPase protein levels and the frequency at which twitch tension was maximum (r=.74, n=18, and P<.001 in the case of normalization per total protein). Similarly, SR Ca\(^{2+}\)-ATPase protein levels were correlated with the potentiation of twitch tension after an increase in stimulation frequency from 30 to 120 min\(^{-1}\) (r=.80, n=18, and P<.001). These data show that the protein levels of SR Ca\(^{2+}\)-ATPase are closely related to the force-frequency behavior of human myocardium. This may suggest that protein levels of SR Ca\(^{2+}\)-ATPase determine the systolic contractile reserve with respect to frequency potentiation of contractile force in the human myocardium. (Circ Res. 1994;75:434-442.)

**Key Words** force-frequency relation • ischemic cardiomyopathy • dilated cardiomyopathy • heart failure • excitation-contraction coupling

The influence of heart rate on myocardial performance has been investigated under in vivo and in vitro conditions. In most species, a positive force-frequency relation has been observed, and frequency potentiation of contractile force has been considered to be a major mechanism to increase myocardial performance during exercise or increased stress.\(^1\)-\(^5\) Recently, existence of a positive force-frequency relation was demonstrated in the nonfailing human heart: (1) In isolated myocardial strips from hearts with normal left ventricular function, it was shown that contractile force increases with increasing rates of stimulation during and above the whole physiological frequency range.\(^6\)-\(^7\) (2) In patients with normal left ventricular function, it was demonstrated that hemodynamic parameters of myocardial contractility increase progressively with increasing pacing rates during atrial or ventricular stimulation.\(^8\)-\(^9\)

However, in contrast to the nonfailing human heart, in the failing heart, frequency potentiation of contractile force was shown to be absent.\(^9\) Moreover, it was observed in isolated human myocardium that in most muscle strips from failing hearts, increasing stimulation frequency results in a progressive depression of isometric tension development.\(^6\)-\(^7,10,11\)

The altered force-frequency relation of failing human myocardium may be related to disturbed excitation-contraction coupling processes at the level of the sarcoplasmic reticulum (SR). Disturbed SR function was suggested in several studies showing that the mRNA expression of the SR Ca\(^{2+}\) pump is reduced in the failing human heart.\(^12,13\) Furthermore, reduced SR Ca\(^{2+}\)-ATPase expression was confirmed at the level of the protein.\(^14\) These findings are in line with data from animal models of myocardial hypertrophy, showing that SR Ca\(^{2+}\)-ATPase is reduced at the level of both the mRNA and the protein.\(^15\)-\(^17\) However, a functional relation between myocardial performance and expression of SR Ca\(^{2+}\)-ATPase has not been demonstrated yet. Moreover, conflicting results were obtained when SR Ca\(^{2+}\) uptake was investigated in homogenates or isolated vesicles of failing human hearts.\(^18-20\)

Accordingly, it was the goal of the present study to investigate the relation between myocardial function as
evaluated by force-frequency behavior and expression of SR Ca\(^{2+}\)-ATPase in nonfailing and failing human myocardium. The force-frequency relation was characterized in isolated myocardial strip preparations under conditions of physiological temperature and stimulation rates between 30 and 180 beats per minute. In myocardium from the same hearts, SR Ca\(^{2+}\)-ATPase expression was evaluated by quantification of protein levels using Western blot analysis. In addition, Ca\(^{2+}\) uptake by SR was measured in myocardial homogenates and related to protein levels of SR Ca\(^{2+}\)-ATPase.

**Materials and Methods**

**Patients**

Analyses of myocardial function and protein levels of SR Ca\(^{2+}\)-ATPase were performed in myocardium from 18 human hearts. Failing myocardium was obtained from explanted hearts of patients with end-stage heart failure due to dilated or ischemic cardiomyopathy who were undergoing cardiac transplantation. The clinical characteristics of the patients are given in Table 1. Four nonfailing hearts were obtained from brain-dead organ donors (patients 1 through 4 in Fig 1): two were female, and two were male; their mean age was 46 ± 6 years. The donor hearts could not be used for cardiac transplantation for technical reasons. No history of cardiac disease was present. The hearts used for the present study were completely different from those used in a previous study from our group.14

The present study was reviewed and approved by the ethical committee of the University Clinics of Freiburg.

**Muscle Strip Preparation**

Immediately after cardiectomy, a portion of the left ventricle was excised and submerged in protective solution at room temperature and oxygenated by bubbling with 95% O\(_2\)/5% CO\(_2\). The solution contained (mmol/L) Na\(^+\) 152, K\(^+\) 3.6, Cl\(^-\) 135, HCO\(_3\)^- 25, Mg\(^{2+}\) 1.6, H\(_2\)PO\(_4\)^- 1.3, SO\(_4^{2-}\) 0.6, Ca\(^{2+}\) 2.5, glucose 11.2, and 2,3-butaneinedione monoxime 30, along with 10 IU/L insulin. This protective solution was shown to preserve human myocardium during transportation and dissection and to be completely reversible after washout.6,21 To prepare the experimental intact muscle strip, the excised myocardium was transferred into a dissection chamber also containing the protective solution. Thin trabeculae were dissected from the endocardial surface of the heart. Alternatively, larger trabecular or papillary muscles were transferred to special dissection chambers, where the myocardium was clamped between the ends of plastic rods and submerged in the protective solution.22 The myocardium could be rotated axially to facilitate dissection, which was performed with microdissection scissors and forceps under microscopic view. Thin muscle strips were dissected parallel to muscle cell orientation as previously described.21,22 In case of the intact trabeculae, a portion of the cross-sectional area consists of noncontractile endocardium. This may explain why tension values were somewhat lower compared with values reported in our previous studies in which muscle strips without endocardium were used. However, in the present study, there were no significant differences in tension generation per cross-sectional area between muscle strips with and without endocardium. Furthermore, a recent study in human atrial myocardium indicated that removal of the endocardium does not significantly influence tension generation of the underlying myocardial tissue.23 Loops of non-capillary braided silk were attached to the end of the preparations with silk ligatures. In some preparations, fine steel hooks were attached to the muscle strips. To perform the mechanical measurements, the muscle was mounted in the muscle chamber and connected to the force gauge (F30 type 372, Hugo Sachs Elektronik; or OPTIL, Scientific Instruments). The muscle was submerged in normal Krebs-Ringer solution at 37°C to wash out the protective solution and stimulated (25% above threshold voltage; duration, 5 milliseconds). After an equilibration period of 30 to 60 minutes, the muscle was stretched gradually (in 0.05- to 0.1-mm steps) to the length at which maximum steady-state twitch force was reached (Lmax). The steady-state isometric force-frequency relation was obtained at 37°C by recording and measuring the twitch after stimulation for 5 minutes at each frequency (30, 60, 90, 120, 150, and 180 beats per minute). Twitch tension, rates of tension rise and fall, and timing parameters were measured from the recordings (WR 3310, Graphtec). Twitch tension is defined as the active tension developed during the isometric twitch. It is the amplitude of the twitch signal between diastolic tension at the end of the stimulus interval and peak systolic tension. Optimal frequency is defined as the

**Table 1. Clinical Characteristics of Patients**

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Age, y</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>EF, % L · min(^{-1}) · m(^{-2})</th>
<th>CI, mm Hg</th>
<th>PCW, mm Hg</th>
<th>Previous Medications</th>
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<td>Furosemide, ISMN, dopamine, dobutamine</td>
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</table>

EF indicates ejection fraction; CI, cardiac index; PCW, mean pulmonary capillary wedge pressure (or left ventricular end-diastolic pressure); DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; and ISMN, isosorbide mononitrate.
frequency at which maximum tension was reached (before a decline of tension occurred) within the frequency range investigated. At the end of each experiment, $I_{max}$ was measured, and the blotted weight of this segment was obtained. Cross-sectional area for normalization of force values was calculated as the ratio of blotted muscle weight to $I_{max}$. Average cross-sectional area of the muscle strips was 0.38 ± 0.04 mm$^2$ (0.11 to 0.63 mm$^2$). These cross-sectional areas have been shown to be below the critical cross-sectional area for adequate oxygenation on the basis of the Paradise protocol performed in previous studies in muscle strips from nonfailing and failing human hearts in our laboratory.24 The assumption of absence of hypoxia as a major cause for frequency-dependent changes in twitch parameters is further supported by the fact that there was no correlation between cross-sectional areas of the muscle strips and optimal frequency or increase in diastolic tension at 180 min within the different groups of myocardium. To ensure adequate oxygen saturation of the organ bath (bubbled with 95% O$_2$/5% CO$_2$) during the mechanical experiments, oxygen partial pressure was measured in the organ bath by an ABL 510 gas analyzer (Radiometer) during a separate set of force-frequency experiments ($n$ = 6). Average oxygen partial pressure was 528 ± 2 mm Hg (range, 511 to 568 mm Hg). Mechanical measurements were performed in 8 left ventricular muscle strips from 4 nonfailing hearts, in 10 muscle strips (8 left ventricular and 2 right ventricular) from 9 failing hearts with dilated cardiomyopathy, and in 7 left ventricular muscle strips from 10 hearts with ischemic cardiomyopathy. If mechanical measurements were available from several muscle strips of the same heart, average values of the respective parameters of the individual heart were calculated and used for the calculation of mean values (Table 2, Fig 3) or of correlations (Figs 4, 5, and 6).

**Western Blot Analysis of SR Ca$^{2+}$-ATPase**

**Protein Preparation**

Myocardium for Western blot analyses and for Ca$^{2+}$ uptake measurements was dissected at the time of explantation of the heart, immediately frozen in liquid nitrogen, and stored at −80°C until use. Care was taken not to use scarred, fibrotic, or adipose tissue, endocardium, epicardium, or great vessels. For protein preparation, ∼100 mg of frozen left ventricular tissue was pulverized by use of a Mikro-Dismembrator II (B. Braun Melsungen Inc) for 10 seconds; the tissue was homogenized in a sevenfold volume of lysis buffer (mmol/L: HEPES 20, EGTA 4, and dithiothreitol [DTT] 1 [pH 7.5]) along with protease inhibitors (0.1 mmol/L leupeptin and 0.3 mmol/L phenylmethylsulfonyl fluoride [PMSF, Sigma Ltd]) and stirred for 45 minutes at 4°C. The homogenate was centrifuged at 100 000g for 60 minutes at 4°C in an L-65 ultracentrifuge (Beckman Instruments Ltd). After centrifugation, the supernatant was carefully removed, and the pellet containing the particulate fraction was resuspended in a sevenfold volume of lysis buffer. The protein concentration of the soluble and particulate fraction was measured in triplicate according to the Bradford method (with ovalbumin used as standard), aliquoted, and stored at −80°C until use. The total yield of protein recovery per gram of wet weight was calculated from the protein content of the particulate and the soluble fraction (see “Results”). The yield of protein per gram weight in the particulate fraction was 64.9 ± 6.3, 76.1 ± 5.9, and 66.8 ± 4.2 mg/g in myocardium from nonfailing hearts, hearts with dilated cardiomyopathy, and hearts with ischemic cardiomyopathy, respectively (no significant differences between groups).

**Western Blot Analysis**

Samples of 100 μg protein of the particulate fraction were denatured by heating to 95°C in 5% sodium dodecyl sulfate (SDS), 13.3% acetic acid, 0.083 mol/L DTT, 0.033 mol/L Tris-HCl, pH 6.8, and 0.0033% bromophenol blue and subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed under reducing conditions on a 7.5% separation gel with a 4% stacking gel in a MiniProtein II cell (Bio-Rad Ltd). Proteins were transferred to a Hybond-ECL nitrocellulose membrane (Amersham Buchler Ltd) by semidy electromblotting with a Fast Blot B33 apparatus (Biometra Ltd) at 5 mA/cm$^2$ for 2 hours by use of 25 mmol/L Tris-HCl, pH 8.3, 150 mmol/L glycine, and 20% methanol as a blotting buffer. The transfer was checked by staining the nitrocellulose membrane with 0.1% Ponceau S solution (Sigma Ltd) prepared in 2.5% acetic acid. The blot was blocked overnight in 5% nonfat milk solution (20 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, and 5% nonfat milk powder) and washed three times for 5 minutes in washing buffer (TBS, 20 mmol/L Tris-HCl, pH 7.5, and 150 mmol/L NaCl). For immunoreaction, the blot was incubated with a 1:3000 diluted mouse-anti-dog slow skeletal (and cardiac) Ca$^{2+}$-ATPase monoclonal IgG, antibody solution25 for 90 minutes (dilution buffer, 20 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, 1% bovine serum albumin [BSA], and 0.1% Tween 20) and washed three times for 1 minute and then three times for 5 minutes in TBS solution. Immunodetected primary antibody against the SR Ca$^{2+}$-ATPase protein was carried out with a 1:70 000 diluted peroxidase-conjugated anti-mouse IgG secondary antibody (Sigma Ltd) for 60 minutes (dilution buffer, 20 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, 1% BSA, and 0.1% Tween 20). The blot was washed again three times for 1 minute and three times for 10 minutes in TBS, then incubated with the ECL-detection reagent (Amersham Buchler Ltd) for 1 minute, and exposed to an X-OMAT AR x-ray film (Kodak Inc) for 0.5 to 1.5 minutes.

**Quantification of Immunoreactive Bands**

The band densities were evaluated by densitometric scanning using a 2202 UltraTecs laser densitometer (LK). To test for the linearity of the immunodetection system, distinct amounts of protein were analyzed. There was a linear relation between protein amount loaded on the gel and the immunoreactive signals of the SR Ca$^{2+}$-ATPase. Each individual value reported represents the mean of two independent determinations. Western blot analysis was also performed in the soluble fraction by using the same protocol. Since no immunoreactivity of SR Ca$^{2+}$-ATPase was found in the soluble fraction, it can be assumed that the total amount of SR Ca$^{2+}$-ATPase present in the myocardium was recovered from the particulate fraction. SR Ca$^{2+}$-ATPase levels were normalized against the total protein recovered from the myocardium and against the β-myosin heavy chain (β-MHC) protein content of the tissue. For normalization against total protein, the arbitrary densitometric units of SR Ca$^{2+}$-ATPase per protein of the particulate fraction were multiplied by the ratio of protein yield in the particulate fraction and total protein. The latter is the sum of protein yield of the particulate fraction and the soluble fraction.

**Slot Blot Analysis of β-MHC**

For each sample of myocardium, a series of protein amounts of the particulate fraction (0.1, 0.2, 0.3, and 0.4 μg of protein in 150 μL TBS buffer) was spotted to a Hybond-ECL nitrocellulose membrane (Amersham Buchler Ltd) by use of a slot blot apparatus (Serva Ltd) and washed twice with 150 μL TBS buffer. Blocking and washing procedures for slot blot analysis, immunoreaction of primary and secondary antibody, and ECL detection were performed as described for Western blot analysis. For primary immunoreaction, a 1:250 diluted monoclonal antibody specific for residues 23 to 29 of the 23-KD S1 tryptic fragment of human β-MHC protein was used.20 Immunodetection of the primary antibody was performed with a 1:70 000 diluted peroxidase-conjugated anti-mouse IgG secondary antibody (Sigma Ltd). Quantification of immunoreactive bands was performed as described for Western blot analysis. For each tissue, there was a linear relation between
the amounts of loaded protein and the immunoreaction signals of β-MHC protein. No specific immunoreaction of β-MHC was found in the soluble fractions.

**SR Ca²⁺ Uptake**

Oxalate-facilitated Ca²⁺ uptake was measured in myocardium from all nonfailing hearts and in 12 of 14 samples of the failing hearts by use of a Millipore filtration system (Millipore Corp.). The assay was performed according to the method described by Bagani and Solorio. The samples of the frozen myocardium were minced with scissors on an ice-cold rubber surface. Homogenization was performed in 10 mL of ice-cold solution containing 25 mM/ L imidazole (pH 7.0) for four 10-second periods in a Polytron homogenizer (PT-K, Kinematica). An aliquot of the homogenate was transferred into the uptake medium containing (final concentration, mM/L) KCl 100, imidazole 40, potassium oxalate 5, MgCl₂ 4.5, Na₂ATP 2.5, and creatine phosphate 3.0, along with 2 IU/mL creatine phosphokinase (pH 7.0). After an equilibration period of 5 minutes (37°C), the assay was started by adding 25 μM/L CaCl₂ (0.185 μCi ⁴⁰Ca²⁺/mL) and 1.5 μM/L EGTA, yielding a free Ca²⁺ concentration of 7 μM/L. Aliquots of the reaction medium (100 μL) were taken after 30, 60, 90, and 120 seconds, filtered through a 0.45-μM Millipore filter, and rapidly washed with ice-cold solution containing 0.6 mol/L KCl, 5 mM/ L Na₂ATP, and 20 mM/L imidazole to stop the uptake. Radioactivity was determined by liquid scintillation spectroscopy. The Ca²⁺ uptake rate was calculated from the slope of the linear regression analysis relating Ca²⁺ uptake to reaction time. Each uptake rate was calculated as the mean of three independent determinations. Protein was determined according to the Bradford method (see above).

**Statistical Analysis**

Data are expressed as mean±SEM. For comparisons within one group of myocardium, the paired t test was applied. Comparisons between the different types of myocardium were performed by nonpaired t test, if appropriate, or by ANOVA followed by the Student-Newman-Keuls test. Correlations were examined by linear regression analysis. A value of P<.05 was accepted as statistically significant.

**Results**

**Relation Between Myocardial Performance and Stimulation Frequency**

In failing myocardium from hearts with dilated or ischemic cardiomyopathy, twitch tension was not significantly different from the value for nonfailing myocardium at the lowest stimulation rate of 30 min⁻¹ (Table 2). However, because of different force-frequency relations in failing and nonfailing human myocardium, at 120 min⁻¹, tension was reduced by 59% in hearts with dilated cardiomyopathy and by 76% in hearts with ischemic cardiomyopathy compared with nonfailing hearts (Table 2). As is shown in Fig 1, with stimulation frequencies of >30 min⁻¹, twitch tension increased in all preparations from nonfailing hearts. Within the frequency range investigated, maximum tension was reached between 90 and 180 min⁻¹ (Fig 1). The average maximum twitch tension was reached at 120 min⁻¹ and was 76% higher than tension at 30 min⁻¹ (Table 2). In the failing myocardium, maximum tension was reached between 30 and 150 min⁻¹ (Fig 1). Tension was highest at 30 min⁻¹ and declined at stimulation rates of >30 min⁻¹ in 4 of 10 muscle strips from hearts with dilated cardiomyopathy and in 4 of 7 muscle strips from hearts with ischemic cardiomyopathy (Fig 1). On the average, there was no significant increase in tension at stimulation frequencies of >30 min⁻¹ in both groups of failing myocardium (Table 2).

As is obvious from Table 2, there was no significant difference in β-MHC protein content between the different types of myocardium. This contradicts the possibility that the depression in tension generation capacity in the failing myocardium may be related to excessive connective tissue replacement of functional myocytes in the muscle strips from failing hearts.

There was an increase in diastolic tension at stimulation rates of >90 min⁻¹ in 6 of 8 preparations from nonfailing hearts, in 7 of 10 preparations from hearts with dilated cardiomyopathy, and in 4 of 7 preparations from hearts with ischemic cardiomyopathy. In those muscle strips exhibiting a rise of diastolic tension, diastolic tension (given as percentage of maximal twitch tension) was increased by 14±3% (150 min⁻¹) and 33±7% (180 min⁻¹) in nonfailing myocardium, by 14±4% (150 min⁻¹) and 19±4% (180 min⁻¹) in myocardium from hearts with dilated cardiomyopathy, and by 9±2% (150 min⁻¹) and 22±5% (180 min⁻¹) in myocardium from hearts with ischemic cardiomyopathy.

Maximum rates of tension rise and fall did not change with increasing frequency in failing myocardium but increased significantly in nonfailing myocardium with higher stimulation rates (Table 2). At 120 min⁻¹ compared with 30 min⁻¹, maximum rates of tension rise and fall were increased by 147% and 130%, respectively, in nonfailing myocardium (Table 2). Frequency-depen-
Myocardial Function and Protein Levels of SR Ca²⁺-ATPase

The total yield of protein recovered per gram of wet weight of tissue was not significantly different in the three types of myocardium; it was 94±8 mg/g in nonfailing myocardium and 117±8 and 106±6 mg/g in myocardium from hearts with dilated and ischemic cardiomyopathy, respectively. SR Ca²⁺-ATPase protein levels obtained by Western blot analysis and normalized per total protein were significantly reduced by 36% in failing compared with nonfailing myocardium (Figs 2 and 3). There was no significant difference between myocardium from hearts with dilated and ischemic cardiomyopathy (Fig 3). SR Ca²⁺-ATPase normalized per β-MHC protein (in densitometric units SR Ca²⁺-ATPase per densitometric units β-MHC) was 16.3±2.2 in myocardium from nonfailing hearts and 11.2±1.1 in myocardium from failing hearts (P<.05). Again, there was no significant difference between myocardium from hearts with dilated (11.0±1.6) and ischemic (11.6±1.7) cardiomyopathy. To test the hypothesis that differences in the force-frequency relation may be related to different expression of SR Ca²⁺-ATPase, the frequency at which maximum tension was reached was within the force-frequency range investigated (before a decline of tension occurred) was plotted versus protein levels of SR Ca²⁺-ATPase, measured in myocardium from the same hearts. Across the different types of myocardium, there was a close positive correlation between the frequency at which maximum tension was reached (optimal frequency) and SR Ca²⁺-ATPase protein levels (Fig 4, Table 3). Most important, the correlation between both parameters was also statistically significant when the

![Graph](http://circres.ahajournals.org/)

**Fig 1.** Graphs showing the force-frequency relation in nonfailing and failing human myocardium. Twitch tension is given in percentage of the value at 30 min⁻¹. The darker line reflects the mean values in each group. The numbers in parentheses indicate patient numbers for the different hearts from which the muscle strips were prepared (see also Table 1). Muscle strips 7 and 11 in the dilated cardiomyopathy group were prepared from the right ventricle. Since mean values were calculated from the percentage data, frequency-dependent changes are slightly different from those of the mean values in Table 2, calculated from the absolute data. In three muscle strips from hearts with ischemic cardiomyopathy, measurements at 180 min⁻¹ were not available. *For graphical reasons the increase in tension of this strip is plotted on one-half scale.

![Western blot analysis](http://circres.ahajournals.org/)

**Fig 2.** Western blot analysis of the sarcoplasmic reticulum (SR) Ca²⁺-ATPase in myocardium from two nonfailing human hearts (NF) and from four hearts with end-stage failing dilated cardiomyopathy (DCM). The position of the SR Ca²⁺-ATPase is indicated as the 100-kD band. The numbers in parentheses correspond to the numbers in Fig 1.

![Bar graph](http://circres.ahajournals.org/)

**Fig 3.** Bar graphs showing protein levels of sarcoplasmic reticulum Ca²⁺-ATPase normalized per total protein recovered per gram wet weight in nonfailing (n=4) and failing (n=14) human myocardium. The failing group includes hearts with dilated cardiomyopathy (DCM, n=9) and ischemic cardiomyopathy (ICM, n=5).
analysis was performed in failing myocardium from hearts with dilated and ischemic cardiomyopathy or from hearts with dilated cardiomyopathy exclusively (Table 3). In Fig 5, the relation between frequency-dependent changes in twitch tension and SR Ca\(^{2+}\)-ATPase protein levels is obvious. For failing and nonfailing myocardium, there was a significant correlation between the change in twitch tension after an increase in stimulation frequency from 30 to 120 min\(^{-1}\) and SR Ca\(^{2+}\)-ATPase protein levels (Fig 5, Table 3). Again, the correlation between both parameters was also highly statistically significant when the analysis was performed in failing myocardium from hearts with ischemic and dilated cardiomyopathy or from hearts with dilated cardiomyopathy exclusively (Table 3).

**SR Ca\(^{2+}\) Uptake**

Ca\(^{2+}\) uptake in homogenates from nonfailing and failing hearts was 2.25±0.52 and 1.45±0.15 nmol per minute per milligram of total protein, respectively.

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**Table 3. Relation Between Force-Frequency Behavior and Protein Levels of Sarcoplasmic Reticulum Ca\(^{2+}\)-ATPase**

<table>
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<th>Myocardial Groups Included in Analysis</th>
<th>SR Ca(^{2+})-ATPase Normalized per Total Protein vs</th>
<th>SR Ca(^{2+})-ATPase Normalized per (\beta)-MHC vs</th>
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<td>Optimal Frequency</td>
<td>Change in Twitch Tension</td>
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<td>(r=0.80, P=0.001)</td>
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<td>DCM (n=9)</td>
<td>(r=0.82, P=0.01)</td>
<td>(r=0.81, P=0.01)</td>
</tr>
</tbody>
</table>

SR indicates sarcoplasmic reticulum; \(\beta\)-MHC, \(\beta\)-myosin heavy chain; change in twitch tension, change in tension development after an increase in the stimulation frequency from 30 to 120 min\(^{-1}\); DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; and \(r\) linear correlation coefficient.
stimulation frequency but reduced significantly in failing human myocardium at higher rates of stimulation.6-26 However, regarding the force-frequency relation of the individual hearts, it is remarkable that the frequency at which maximum tension was reached (the optimal frequency) varied considerably, even within the same disease group (Fig 1).

In a previous study of force-frequency relations in human myocardium, acetylstrophanthidin added to the organ bath at a concentration of 4×10⁻⁷ mol/L resulted in a depression of the force-frequency relation in both failing and nonfailing myocardium.10 Therefore, some of the depression of the force-frequency curves in failing myocardium might be attributable to the long-lasting effects of digitalis. However, we do not think that the effects of digitalis are a major cause of the altered force-frequency relation in failing human myocardium, because in five muscle strips from three failing hearts not pretreated with digitalis (patients 5, 15, and 18 in Table 1 and Fig 1), optimal frequency was reached at the lowest stimulation rate in three preparations and at 60 and 90 min⁻¹ in one preparation.

To investigate the hypothesis that differences in optimal frequency between failing and nonfailing myocardium and the variation within the failing group may be related to different expressions of SR Ca²⁺-ATPase, protein levels of SR Ca²⁺-ATPase were measured in the same hearts. Again, there was a considerable variation of SR Ca²⁺-ATPase levels even within one group of myocardial preparations. Values differed in the nonfailing group by a factor of two and in the failing group by a factor of five. However, on average, protein levels of SR Ca²⁺-ATPase were reduced significantly in failing compared with nonfailing human myocardium by 36% when normalization was performed per total protein recovered per gram of myocardium and by 32% when normalization was performed per protein levels of β-MHC quantified in the same tissue. The latter normalization indicates that, on average, expression of the SR Ca²⁺ pump is reduced relative to the expression of contractile proteins in the failing human myocardium. The finding of reduced SR Ca²⁺-ATPase protein levels is consistent with (1) recent measurements showing that expression of SR Ca²⁺-ATPase is reduced at the level of the mRNA and the protein in failing compared with nonfailing human myocardium12-14 and (2) Ca²⁺-uptake measurements performed in homogenates of human myocardium, which showed decreased SR Ca²⁺ uptake in the failing compared with the nonfailing preparations.18 Furthermore, in the present study, a close correlation between Ca²⁺ uptake and protein levels of SR Ca²⁺-ATPase was demonstrated. However, it was previously reported that reduced Ca²⁺ uptake was not seen when measurements were performed in highly purified SR vesicles.19

For nonfailing and failing myocardium, there was a significant correlation between optimal frequency and protein levels of SR Ca²⁺-ATPase. Most interesting, this correlation was also highly significant when the analysis was performed in failing myocardium exclusively. In other words, the variation in the force-frequency behavior within the group of failing myocardium matches closely with the variation seen in the protein levels of SR Ca²⁺-ATPase.

The functional relevance of the relation between optimal frequency and SR Ca²⁺-ATPase protein levels is evident when frequency-dependent changes in contractile force (from 30 to 120 min⁻¹) are plotted versus SR Ca²⁺-ATPase. The close correlation between these parameters shows that in myocardium with a low expression of SR Ca²⁺-ATPase, twitch tension decreases when stimulation frequency is increased >30 min⁻¹, whereas in myocardium with a higher expression of SR Ca²⁺-ATPase, contractile force increases considerably at higher rates of stimulation. The validity of this analysis is further supported by the close correlations of Ca²⁺ uptake measured in myocardium from the same hearts with protein levels of SR Ca²⁺-ATPase on the one hand and force-frequency behavior of the strip preparations on the other.

Of course, the close correlation observed between the force-frequency relation and protein levels of SR Ca²⁺-ATPase does not prove a causal relation; therefore, it cannot be excluded that the reduction in SR Ca²⁺-ATPase levels reflects a response to another process primarily causing the altered force-frequency relation in the failing myocardium. However, we think that the highly significant correlation between myocardial function and SR Ca²⁺-ATPase protein levels, and in particular the close relation of both parameters within the group of failing myocardium, may indicate that the expression of SR Ca²⁺-ATPase determines the contractile reserve of the myocardium with respect to frequency potentiation of contractile force.

Assuming a causal relation between the expression of SR Ca²⁺-ATPase and myocardial function, this causality could be explained by the following mechanisms: In myocardium with a high expression of the SR Ca²⁺ pump, the increase in twitch tension at higher rates of stimulation may result from an increased amount of Ca²⁺ released from the SR and available for activation of contractile proteins. Increased Ca²⁺ release at higher stimulation frequencies may be the consequence of a larger amount of Ca²⁺ entering the cell per unit of time and an increased loading of the SR in the presence of a high capacity for SR Ca²⁺ accumulation.29 In myocardium with a low expression of the SR Ca²⁺ pump, the
decrease in twitch tension at higher rates of stimulation may result from a decreased amount of Ca\(^{2+}\) released from the SR. The reduced Ca\(^{2+}\) release may occur because the higher stimulation frequency reduces the time available for Ca\(^{2+}\) transport, which in the presence of a reduced Ca\(^{2+}\) transport capacity may cause SR Ca\(^{2+}\) depletion.

A decreased amount of systolic Ca\(^{2+}\) for activation of contractile proteins as the cause of reduced tension development is consistent with recent myothermal measurements showing that the total amount of Ca\(^{2+}\) cycling is reduced at physiological stimulation rates in the failing human myocardium. In addition, reduced peak systolic Ca\(^{2+}\) concentrations were suggested from fura 2 measurements in isolated myocytes from failing human hearts.

In addition to an insufficient capacity of the SR to accumulate Ca\(^{2+}\) at higher stimulation frequencies, decreased expression of the SR Ca\(^{2+}\) -release channel may be involved in the pathogenesis of the altered force-frequency relation in the failing human heart. This would be consistent with the recent report of a parallel decrease in mRNA levels of the SR Ca\(^{2+}\) -ATPase and the Ca\(^{2+}\) -release channel in failing human myocardium. On the other hand, in a recent study by Brillantes et al., mRNA levels of the Ca\(^{2+}\) -release channel were reduced in myocardium from hearts with ischemic cardiomyopathy but not in hearts with dilated cardiomyopathy.

If an insufficient capacity of the SR to accumulate Ca\(^{2+}\) at higher rates of stimulation were the cause of reduced systolic Ca\(^{2+}\) release and thus reduced tension development, one would expect increased diastolic Ca\(^{2+}\) levels to be the consequence. The latter, in turn, would result in diastolic activation of contractile proteins and thus would cause a rise in diastolic tension. However, since even at the highest stimulation frequency, a rise of diastolic tension was not consistently observed in the failing human myocardium, alternative mechanisms to remove Ca\(^{2+}\) from the cytosol and prevent diastolic activation of contractile proteins must exist. Minor changes in diastolic tension despite a considerable decline in systolic tension, as observed here, are consistent with a recent analysis of frequency-dependent alterations of diastolic function in isolated failing human myocardium. Instead of Ca\(^{2+}\) removal by the SR, Ca\(^{2+}\) could be removed from the cytosol by mitochondria, which have the potential to accumulate high amounts of Ca\(^{2+}\) and have been suggested to be involved in the control of cellular Ca\(^{2+}\) homeostasis. Furthermore, Ca\(^{2+}\) could be buffered by different intracellular Ca\(^{2+}\)-binding proteins such as troponin C or calmodulin. Finally, Ca\(^{2+}\) could be extruded into the extracellular space by sarcolemmal transport mechanisms such as the sarcolemmal Ca\(^{2+}\) -ATPase or the Na\(^+\)-Ca\(^{2+}\) exchanger. The latter mechanism is of particular interest since it was shown recently that expression of the Na\(^+\)-Ca\(^{2+}\) exchanger is increased at the level of the mRNA as well as the protein in failing human myocardium. Of course, Ca\(^{2+}\) extruded into the extracellular space by the Na\(^+\)-Ca\(^{2+}\) exchanger is no longer available for the activation of contractile proteins during systole.

In summary, the present study indicates that there is a close relation between the expression of SR Ca\(^{2+}\) -ATPase and myocardial function. This is consistent with the concept that the reduced expression of SR Ca\(^{2+}\) -ATPase is involved in the altered force-frequency relation of failing human myocardium. It should be stated again that the present finding of a statistical correlation between SR Ca\(^{2+}\) -ATPase protein levels and force-frequency behavior of the myocardium does not prove the existence of a mechanistic relation. It is conceivable that other Ca\(^{2+}\)-handling proteins, the expression of which may be altered in the failing heart, contribute to the force-frequency behavior of the human myocardium. Therefore, further studies that evaluate the effects of specific inhibition or activation of SR Ca\(^{2+}\) -ATPase on the force-frequency behavior of human myocardium are warranted.

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Relation between myocardial function and expression of sarcoplasmic reticulum Ca(2+)-ATPase in failing and nonfailing human myocardium.

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