Special Article

A Mechanistic Analysis of Reduced Mechanical Performance in Human Heart Failure

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SUMMARY

In failing human hearts (FHH) (NYHA IV) the cardiac output is inadequate to meet the metabolic needs of the peripheral systems. By means of thermo-mechanical analysis we have shown that epicardial strips from FHH (37 °C) have a depressed tension independent heat (TIH) and tension independent heat rate (dTIH / dt) liberation that correlates with depression in peak isometric force and the rate of relaxation. Furthermore, in response to a change in frequency of stimulation, FHH shows a severe blunting of the force-frequency relationship resulting in a decrease in myocardial reserve and in the frequency at which optimum force is obtained. We used ventricular ANF as an index of the severity of myocardial disease and demonstrated an inverse relationship between ANF mRNA and the sarcoplasmic reticulum (SR) calcium cycling proteins (SERCA 2, Phospholamban, Ryanodine Receptor) while these latter proteins all had a positive correlation with each other. At the same time there was an increase in sarcolemmal sodium calcium exchange protein. The decrease in SR pump proteins correlates with the decrease in myocardial reserve and optimum frequency of contraction. The latter mechanical changes are explainable in terms of a frequency dependent decrease in calcium concentration (aequorin light) in FHH. (Jpn Heart J 2000; 41: 103-116)

Key words: Peak isometric force, Isometric relaxation rate, Calcium cycling, Force frequency relationship, Heat production, SERCA 2, Ryanodine receptor, Phospholamban, NaCa exchange protein, Ventricular ANF

IN human heart failure the cardiac output is inadequate to meet the metabolic needs of the peripheral tissues. The severity of the failure varies from very

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severe to mild and has been defined by the New York Heart Association as follows: 1) In the severest failure, Class IV, symptoms such as shortness of breath and fatigue are present at rest; 2) In Class III failure symptoms are prominent with activity of daily living; 3) For Class II failure, symptoms become noticeable only with increased level of mild activity; and 4) Patients in Class I failure exhibit symptoms with severe activity. In all of these cases there are observable alterations of the mechanical performance of the heart which include a reduction in the peak isometric force developed, the rate of isometric relaxation, the myocardial reserve and the optimum contraction frequency. In this review the experiments and data presented are chosen to elucidate the mechanisms underlying the depression in myocardial mechanics found in the failing heart. For the purpose of clarity we have chosen to compare tissue samples from non failing and severely failing (NYHA Class IV) human hearts.

METHODS

Human myocardial biopsy and dissection procedures: Strips from the sub-epicardium were obtained from patients undergoing coronary artery bypass surgery or immediately following cardiac transplantation. The non failing heart tissue was obtained from coronary artery bypass patients with normal left ventricular function (*i.e.* ejection fraction greater than 60 %). Failing myocardium was obtained from hearts explanted from patients suffering from idiopathic dilated cardiomyopathy (NYHA Class IV failure; mean left ventricular ejection fraction, 13±1 %). The epicardial preparations from the non failing hearts were obtained shortly after cardioplegic arrest.[1)*] There were no complication in any patient from the biopsy procedure. Immediately after the epicardial strip, consisting of parallel running fibers, was dissected free from the heart, it was placed in the BDM protective solution consisting of Krebs-Ringer solution plus 30 mmoles / *l* of 2,3-butanedione monoxime.[1)] The tissue was allowed to incubate in the oxygenated protective solution for one hour to permit recovery from the trauma of surgical biopsy. Thin strips were then dissected from the original biopsy material so that the final preparation was approximately 0.3 mm in diameter.[1)] Tissues were kept in oxygenated (95 % O_2 / 5 CO_2) Krebs-Ringer solution after washing out the BDM protective solution.

Force and myothermal measurements: All measurements were made at 37 ℃. The peak tension for isometric contraction was measured in each muscle strip at the optimum of the tension length relation using methods previously des-

* Patients gave infomed consent. The study was approved by the Committee on Human Research of the University of Vermont.

cribed.[2-4] The steady state tension-frequency relation was obtained with 5 minutes of stimulation at each frequency starting at 0.2 Hz and increasing in 0.2 Hz increments. The cross-sectional area was obtained from the quotient of the length at L_{max} (3 - 4 mm) and the blotted weight of the active portion of each muscle strip (0.2 - 0.6 mg). The apparatus and protocols for carrying out mechanical-myothermal measurements on thin strips of human heart tissue have been described in detail elsewhere.[5] The carefully dissected muscle is positioned on the centrally located heat measuring junctions of the thermopile and incubated in normal Krebs-Ringer solution for 90 minutes to thoroughly wash out the BDM-Ringer protective solution. The muscle strips were mounted on the thermopile so that the flat end of the muscle was apposed to the measuring junctions with the top end of the strip attached to a force transducer while the bottom end was fixed to a stationary hook. The washout solution is completely replaced with fresh oxygenated Ringer (95 % O_2 / 5 % CO_2) and the muscle stimulated (stimulating electrodes of 25 μm platinum wire are imbedded in the 4-0 noncapillary braided silk used to tie the muscle to the force transducer and fixed hook) while being stretched in small increments (0.05 mm) until maximum active force is developed.

The chamber with the muscle is then drained of all solutions and the measurements of force and heat production are made.

Analysis of the mechanical and myothermal measurements: When the strip is stimulated, the muscle contracts and then relaxes, developing an isometric force

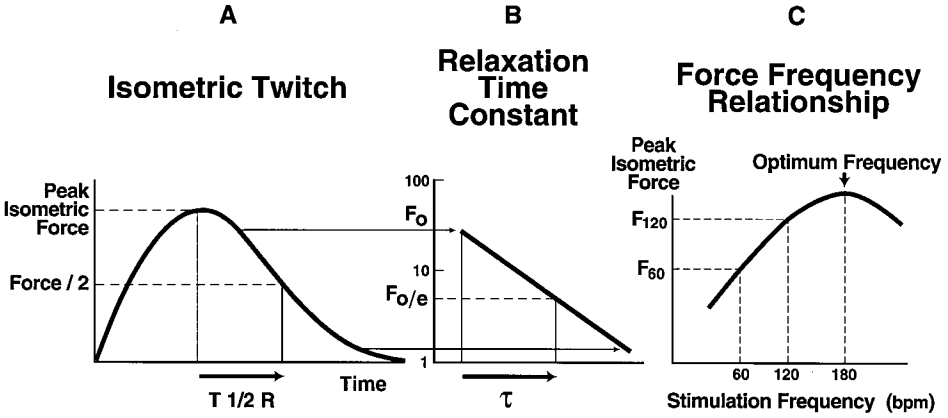

Figure 1. Isometric force, relaxation rate, myocardial reserve and optimum contraction frequency. In panel A the time course of isometric force is presented. Peak isometric force and time to one half relaxation (T ½ R) are indicated on the myogram. The relaxation time constant (time from F_o to F_o / e) is illustrated in panel B. In panel C the myocardial reserve is defined as the force at 120 Hz (F_{120}) divided by the force at 60 Hz (F_{60}) multiplied by 100 while the optimal frequency is defined as the frequency at which optimal force occurs.

myogram (Figure 1). The peak force is the maximum isometric force developed by the muscle strip (Figure 1A) while isometric relaxation is characterized in terms of $T_{1/2}R$ or the relaxation time constant (Figure 1A, B). Myocardial reserve is the percent increase in force between stimulation frequencies of 60 and 120 Hz while the optimal frequency is the frequency of stimulation at which maximum force occurs (Figure 1 C). A strip of heart muscle at rest liberates heat at a steady rate. This heat production is designated the resting heat and it represents the energy requirements for maintaining the internal milieu as well as housekeeping protein synthesis. When a muscle is stimulated there is a rapid evolution of heat followed by a secondary slower rate of heat liberation. The latter is liberated at a monoexponentially decreasing rate. This heat production is the total activity related heat production (T_A). The secondary slower rate of heat production is the recovery heat R which is a reflection of the mitochondrial resynthesis of ATP from the ADP released during contraction and relaxation. Subtracting the recovery heat (R) from the total activity related heat (T_A) leaves the initial heat (I) (Eq 1). The initial heat represents the energy liberation resulting from myosin cross-bridge cycling and calcium cycling. The initial heat (I) can be partitioned into a tension dependent component (TDH)(cross-bridge cycling) and a tension independent component (TIH) associated with calcium cycling (Eq 2). The tension independent heat (TIH), along with the enthalpy of CrP hydrolysis, can be used to calculate the amount of calcium cycled per gram of muscle during each contraction-relaxation cycle (Eq 3). The Ca^{++} :CrP coupling ratio is assumed to be 2 for sarcoplasmic reticulum transport[6] while K is a factor that takes into consideration factors other than the sarcoplasmic reticulum that contribute to TIH.[7] A detailed discussion for the rationale and background for this analysis and these measurements is presented elsewhere.[2,5-9]

$$T_A - R = I \quad \text{(Eq. 1)}$$

$$I = TDH + TIH \quad \text{(Eq. 2)}$$

$$Ca^{++}/\text{gm-cycle} = K\,(TIH/gm)/\,[34kJ/\text{ mole X } Ca^{++}\text{: CrP coupling ratio}] \quad \text{(Eq. 3)}$$

Protein and mRNA measurements: Detailed descriptions of the specific protein and mRNA measurements have been previously presented.[5,10-15]

Statistical analysis: Differences between group means were assessed using an unpaired t test where $p < 0.05$ was considered to be significant. Correlation coefficients were calculated using standard analysis of variance.

Results and Discussion

Mechanical and Myothermal Measurements: Peak isometric force in the non

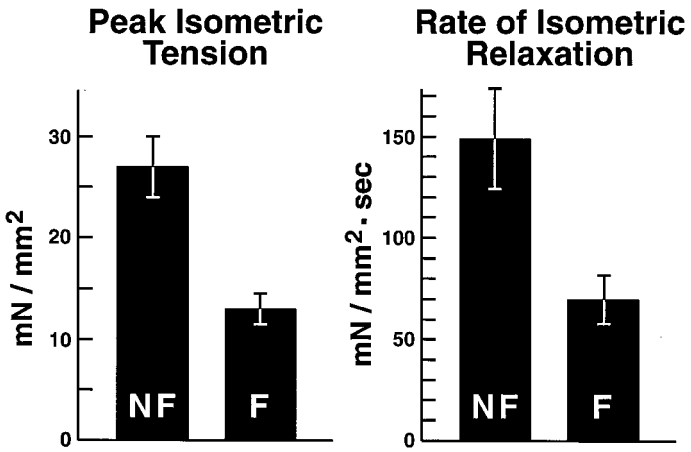

Figure 2. Peak isometric force (left hand panel) and relaxation rate (right hand panel) in non failing (NF) and failing (F) myocardium. The bars represent SEM.

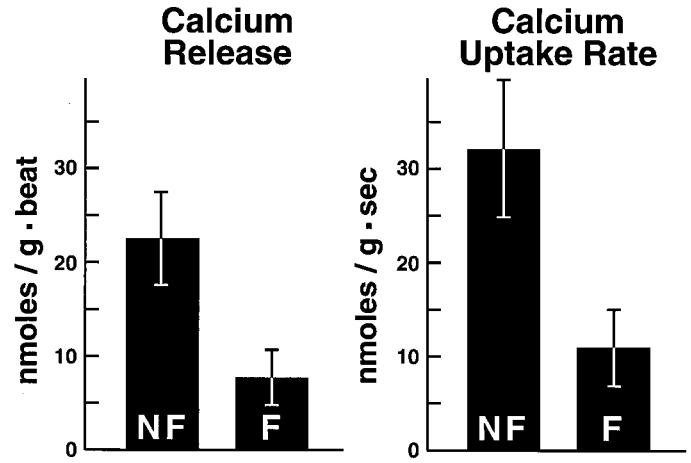

Figure 3. Calcium release and uptake rate in non failing (NF) and failing (F) myocardium. The bars represent SEM.

failing muscle strips was 27.3 ± 3 mN / mm^2. In failing hearts peak isometric force was reduced 51% ($p < 0.02$) (Figure 2). Time to peak tension was prolonged in the failing preparation (NF, 189 ± 9 msec; F, 216 ± 8 msec; $p < 0.03$). The rate of relaxation for the non failing muscle strip was 149 ± 25 mN / mm^2-sec which was reduced 53 % in the strips from failing hearts ($p < 0.02$) (Figure 2). The initial heat production (I) for non failing and failing heart strips was 3.89 ± 0.66 and 1.50 ± 0.26 mJ / gm-beat ($p < 0.005$), while the tension independent heat liberated was 0.51 ± 0.13 and 0.16 ± 0.05 mJ / gm-beat ($p < 0.03$), respectively. From the TIH the calcium cycled per beat and the rate of

calcium removal from the cytosol can be calculated (Eq. 3). In non failing epicardial strips the amount of calcium introduced into the cytosol per beat was 22.5 ± 5.1 nmoles / gm while the uptake rate was 32.1 ± 7.7 nmoles / gm-sec. In the strips from the failing preparations the calcium released into the cytosol was reduced 66 % ($p < 0.03$) while the uptake rate was reduced 65 % ($p < 0.03$) (Figure 3).

Isometric peak force, isometric relaxation rate, calcium release and calcium uptake rate: There is a substantial depression in peak isometric force in failing heart preparations. This depression in force is readily attributable to an inadequate release of calcium following sarcolemmal depolarization and thus a decrease in the degree of activation. The relationship between isometric peak force and calcium release is presented in Figure 4. The depressed force and the diminished calcium release correlate very well (Figure 4). In addition to the changes in force, all of the kinetic parameters of the isometric contraction-relaxation cycle are diminished in the strip preparations from the failing heart. The relaxation rate is reduced by 65 %. Following activation of the myocytes, relaxation depends on the calcium removal from the cytosol. Figure 5 shows the relationship between isometric relaxation and the rate of calcium removal from the cytosol.

The force frequency relation and myocardial reserve: In normal hearts when the frequency of stimulation is increased there is an increase in isometric force until a maximum is reached (optimal frequency). Increases in frequency of stimulation beyond this point result in a decrease in force development (see NF Figure 6). The myocardial reserve of the heart is defined as the percent increase in force as the heart rate is increased from 60 to 120 beats per minute (Figure 1). From the

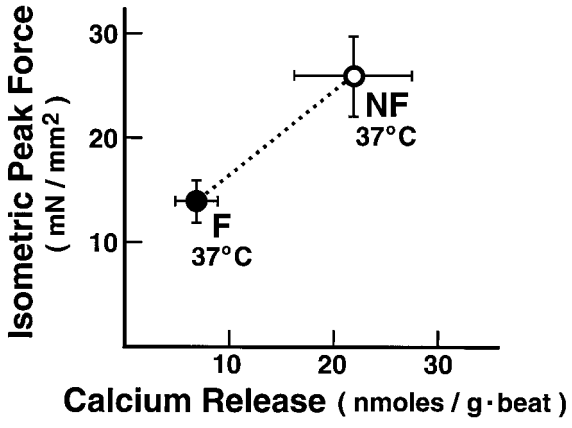

Figure 4. Peak isometric force versus calcium release per beat in the non failing (NF) and failing (F) strip preparations. The bars represent SEM.

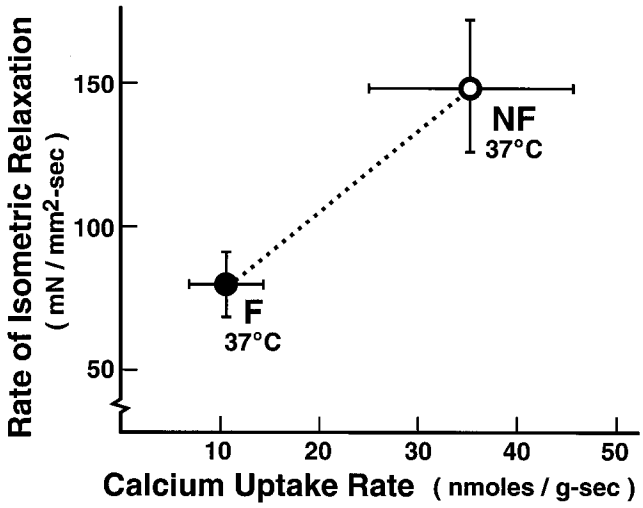

Figure 5. The rate of isometric relaxation versus the rate of calcium uptake in the epicardial strips from non failing (NF) and failing (F) hearts. The bars represent the SEM.

diagram for the non failing preparations (Figure 6) it is clear that the non failing heart has a substantial myocardial reserve with the optimum frequency of 177 ± 4 min^{-1}. In contrast the failing preparation has a negative myocardial reserve (namely a decrease in force between a stimulation frequency of 60 and 120 min^{-1}) with an optimum frequency of 81 ± 22 min^{-1}.[4] The efficacy of assessing ventricular function from myocardial strips is supported by the finding of good agreement between in vitro rate treppe obtained from epicardial strips from non failing hearts and ventricular function curves obtained by others from non failing hearts *in vivo*.[16] The blunting of the force frequency relationship with the reduction in optimal frequency of contraction and ultimate development of a negative myocardial reserve plays an important role along with the reduction in absolute force development in the sequel leading to congestive failure.

The force-frequency relationship and calcium cycling: In view of the close relationship between isometric force development and the amount of calcium released into the cytosol per gm of heart muscle per beat (Figure 4), it is reasonable to believe that this relationship between force developed and calcium cycling can be extended to account for the alterations seen when the frequency of stimulation is increased. This hypothesis was tested in non failing and failing hearts by use of the aequorin intracellular calcium detecting technology.[17] When intracellular calcium concentration is measured in non failing (NF) preparations the aequorin light signal and the peak isometric force rise and fall correlatively (Figure 7, left hand panel). Under these circumstances the peak force and peak

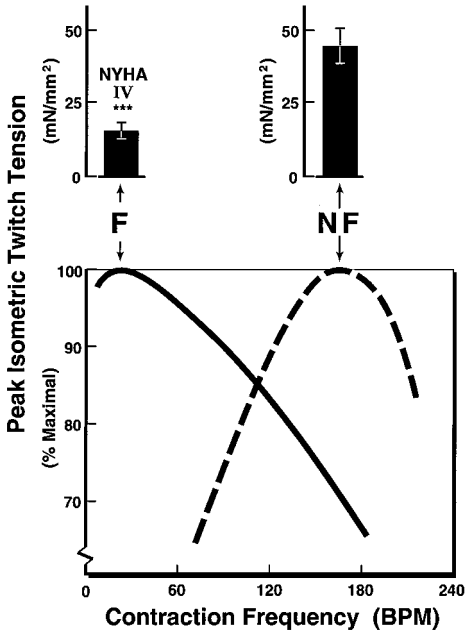

Figure 6. The force frequency relationship in the non failing (NF) and failing (F) preparations. The isometric force developed by the failing (F, NYHA IV) and non failing (NF) strip preparations is presented in the upper portion of the diagram. The bars indicate SEM. These force-frequency values are normalized to 100% for the force developed at optimal frequency in order to diagram the force frequency relationship for the failing (F) and non failing (NF) preparations (bottom portion of the diagram).

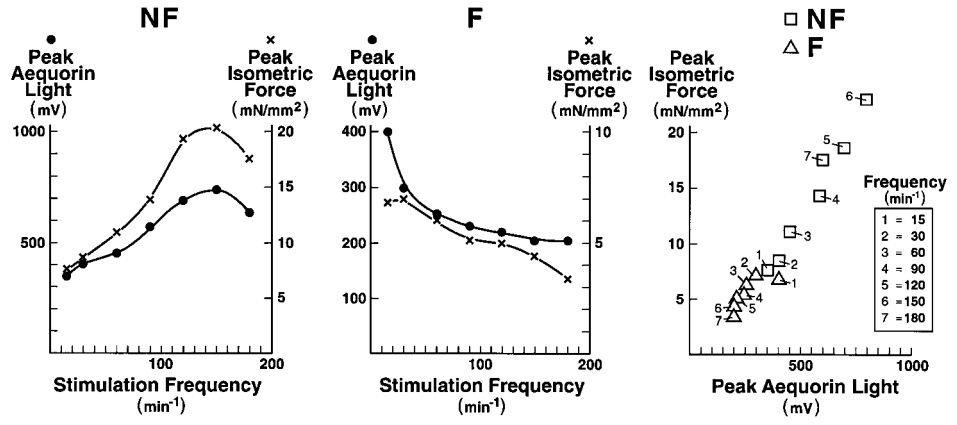

Figure 7. The frequency dependence of peak aequorin light and peak isometric force in non failing (NF)(left panel) and failing (F) prepurations (center panel). In the right hand panel peak isometric force is plotted against the peak aequorin light signal. The numbers in the squares and triangles provide a key for the frequency at which the preparations were stimulated (Redrawn with permission from 18).

light signal rise until the optimal frequency is reached (174 min^{-1}) and then there is a decline in both. In contrast, for the failing (F) preparations there is a persistent decline in both force and aequorin light signals as the frequency of stimulation is increased from very low levels to higher levels (Figure 7, center panel). We posed the question as to the possibility that the sensitivity of the contractile system in the failing hearts might be substantially reduced and that this reduction in sensitivity would contribute to the marked blunting of the force-frequency relationship. When peak isometric force for non failing and failing preparations is plotted against peak aeqourin light, there is a linear relationship with no evidence that the sensitivity of the relationship (slope) for the failing hearts is different from that of the non failing preparations (Figure 7, right hand panel).

The calcium handling proteins: The major alteration in the quantity of calcium released into the cytosol following depolarization of the sarcolemmal membrane and the decreased rate of calcium uptake in failing preparations (Figures 2 and 3) suggest that there are changes in the myocardial calcium cycling proteins. Evidence for a decrease in the SERCA 2 pump is found in measurements of pump protein relative to total protein or pump protein relative to phospholamban where these values are depressed 40 % and 25 %, respectively.[15] The depression in the SERCA 2 message as well as pump protein in failing human hearts has been well documented.[13,15,19-23] Along with this depression in SERCA 2 pump protein it was shown that in failing hearts, where the severity of failure was assessed by ventricular ANF mRNA levels, there is a correlation among SERCA2 calcium pump ATPase, phospholamban as well as ryanodine receptor mRNA (Figure 8).[11] Thus it would appear that in failing hearts all of the sarcoplasmic reticular calcium cycling proteins are down regulated and this observation has been supported by a number of additional measurements[21,24,25] (Figure 9). In contrast to the down regulation of the calcium cycling proteins in the sarcoplasmic reticulum, there is an up regulation of the sarcolemmal sodium-calcium exchange protein where the mRNA level normalized for 18S mRNA and the protein normalized per myosin heavy chain is increased by 83 % ($p < 0.05$) and 85 % ($p < 0.05$), respectively[17] (Figure 9). These findings have recently been confirmed in other laboratories.[26,27] The depression in the sarcoplasmic reticular calcium cycling proteins and the increase in the sarcolemmal sodium calcium exchange proteins results in a diminished release of calcium for activation at all frequencies for the failing hearts.

Correlation of myocardial reserve and peak isometric force with calcium cycling protein levels: From the observations on calcium cycling proteins it would appear that sarcoplasmic reticular calcium ATPase, phospholamban and ryanodine receptors are down regulated in the human heart failure while there is an up

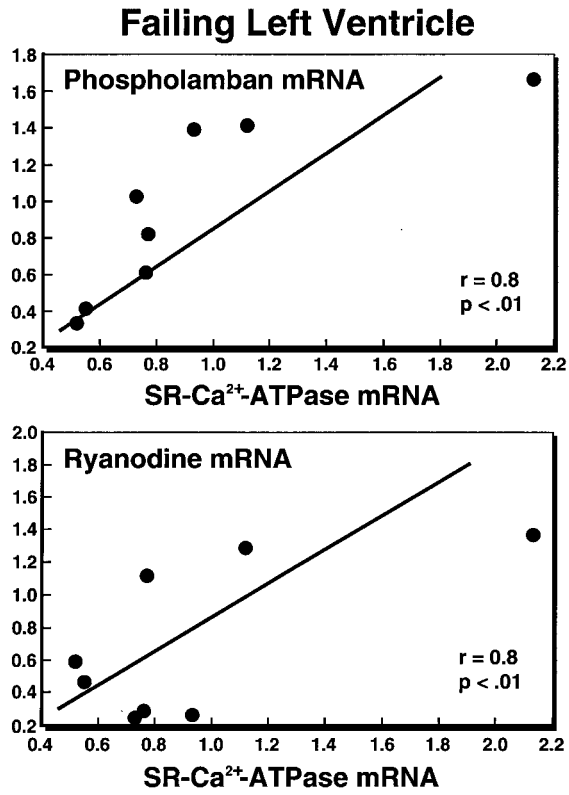

Figure 8. Correlation of phospholamban mRNA (upper diagram) and ryanodine receptor mRNA (lower diagram) with sarcoplasmic reticulum calcium ATPase mRNA (redrawn from 11).

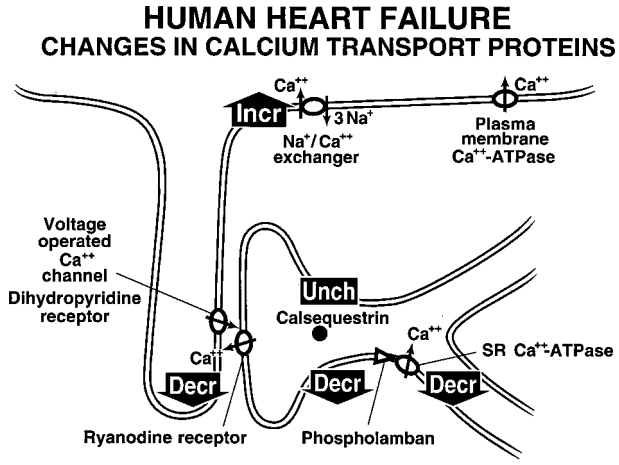

Figure 9. Diagram of the changes in the calcium cycling proteins in the sarcoplasmic reticulum and sarcolemma found in failing hearts.

regulation of the sarcolemmal sodium calcium exchange protein (Figure 9). This constellation of changes results in a decrease, for failing hearts, in the amount of calcium released following sarcolemmal membrane depolarization at all frequencies of stimulation (Figure 7). In order to examine this phenomenon further, the myocardial reserve or increase in force between two different frequencies of stimulation and the optimal frequency of contraction were plotted against the sarcoplasmic reticular calcium ATPase (Figure 10).[19] The correlation coeffi-

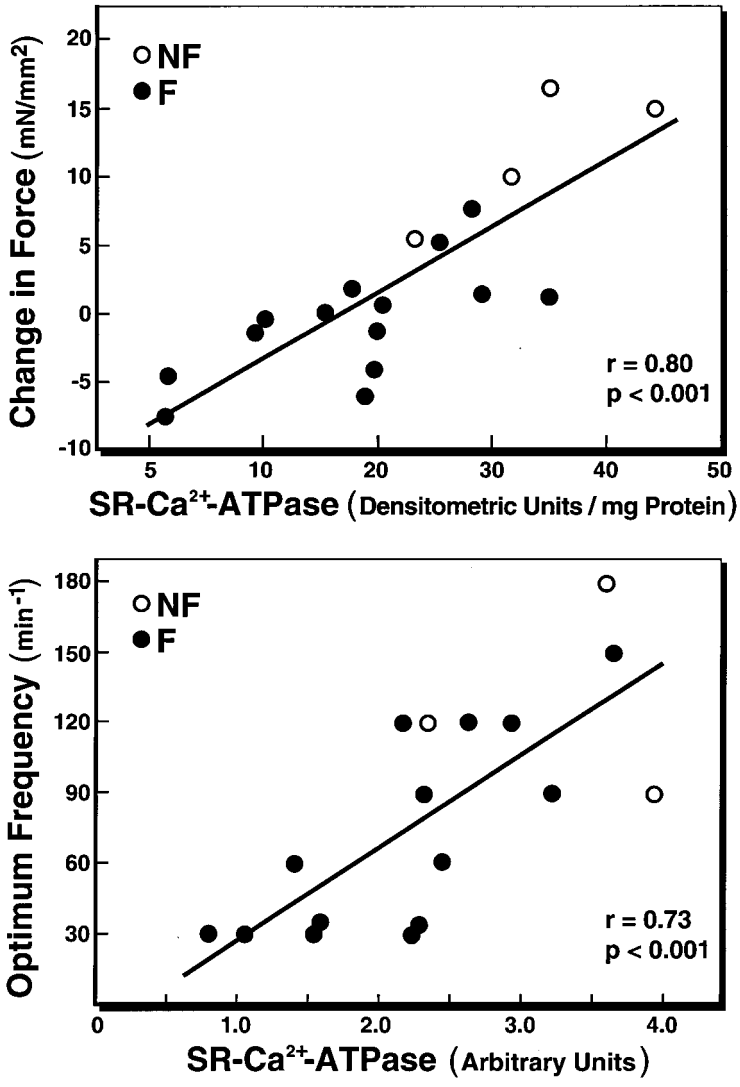

Figure 10. The relationship between myocardial reserve (change in force with change in frequency of stimulation) and optimal frequency with an index of sarcoplasmic reticulum calcium ATPase (redrawn from 19).

cients for change in force and optimum frequency are 0.80 ($p < 0.001$) and 0.73 ($p < 0.001$), respectively (Figure 10).[19)]

Conclusions: A new method for protecting strips of heart muscle during fine dissection and transport of tissues from transplantation centers has been described. This permits mechanical and myothermal measurements to be carried out at 37 °C. We have shown evidence for dramatic depression in mechanical performance in strips from failing heart muscle. This alteration in mechanical performance (isometric force, isometric relaxation rate, myocardial reserve, optimal frequency of contraction) shows a strong correlation with the amount of calcium released following activation and the rate of calcium uptake. There is also a very strong correlation with the quantity of calcium released and calcium uptake kinetics and documented decreases in the sarcoplasmic reticular calcium cycling proteins (SERCA 2, phospholamban, ryanodine receptor) and the increase in the sarcolemmal sodium calcium exchanger. One should be cautious in interpreting data based on correlations. Correlations do not prove cause and effect. Additional experiments are required before we can be certain about the nature of cause and effect in failing hearts. One can propose, however, that animal models of heart failure should include the constellation of changes described above for the failing human hearts.

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