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Creatine and Phosphate Pools are Maintained at Energetically Optimal Levels in the Heart During Hypertrophic Remodeling and Heart Failure

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Abstract— **The ability of mitochondria to oxidatively synthesize ATP from ADP and inorganic phosphate is compromised in the failing heart. Specifically, the magnitude of the free energy at which ATP is synthesized in heart failure is diminished compared to control. However the causal mechanisms involved are not clearly understood. Here we used computer simulation to analyze the impact of reduction in three cytoplasmic metabolic pools that is observed with hypertrophic remodeling and heart failure. Our simulations, which are validated based on *in vivo* data on phosphate metabolites in both the healthy and diseased heart, predict that, given a prescribed reduction in the total adenine nucleotide pool, the pools of total creatine and total exchangeable phosphate are maintained at levels that maintain the ATP hydrolysis potential of the heart at near the normal physiological value.**

I. INTRODUCTION

A. Metabolic Remodeling in Heart Disease

Mechanical work in the failing heart is limited by the free energy at which mitochondria can synthesize ATP [6]. Mitochondrial content and oxidative capacity of cardiac muscle cells may be diminished in heart failure [1], [2]. Key metabolic pools-including creatine, adenine nucleotides, and the total phosphate pool are diminished in heart failure [2], [3]. The result of these metabolic remodeling processes is that chemical energy, in the form of the ATP hydrolysis potential, available for the heart to do work is diminished [4], [5]. Thus the altered metabolic pattern observed in heart failure has a clear impact on the energetic state of the heart: the potential consequences of a diminished energetic state include an impaired ability of the heart to work and respond to acute and chronic stresses.

Experimental observations on failing human hearts and on animal models of hypertrophic remodeling and heart failure have shown that the total adenine nucleotide and creatine pools are reduced in these disease states. We have recently shown, based on analyzing data from a canine model of left-ventricular hypertrophy (LVH) and heart failure, that the total phosphate pool is reduced as well in that setting [11]. Furthermore, we used computer simulation to evaluate the effect of the gradual loss of certain metabolic pools observed in heart failure on the ability of mitochondria to maintain the energetic state of the heart. We showed that the two clinically observed stages of metabolic remodeling—compensatory followed by maladaptive failure—occur as a consequence of the loss of these metabolic pools in the canine model of LVH. Thus our simulations predicts that the observed remodeling of those metabolic pools is associated with a critical phenomenon, where low and moderate reductions have no negative impact on metabolic state while reductions beyond a critical tipping point lead to a severely compromised state.

B. Creatine and Phosphate Pools May be Regulated at Optimal Physiological Levels with Respect to Adenine Nucleotide Pool

Although the reduction in these three metabolic pools beyond the critical point leads to a drastic impairment of energetic state—reflected in a reduction in the magnitude of the free energy for ATP hydrolysis—our previous analysis predicts that the phosphate and creatine pools may attain values that are nearly optimal, given a value of the adenine nucleotide pool. Specifically, the physiologically optimal levels of these pools are defined here as the levels at which the magnitude of the ATP hydrolysis potential remains at or above the normal physiological level. Thus our hypothesis is that, for a given value of the total adenine nucleotide pool (TAN), the total creatine (CR_{tot}) and the total exchangeable phosphate pool (TEP) change to obtain values for which the free energy of ATP hydrolysis remains close to the physiological level.

Indeed, Wallis et al. [8] report that the phosphate and creatine pools are maintained in a "fine balance" in the healthy heart. Our analysis predicts that this fine balance is maintained during disease remodeling in the face of reductions in TAN. Thus one possible explanation for the observed phenomena is that reductions in TAN drive reductions in the other pools during hypertrophy and heart failure. In this study we investigate predictions associated with this hypothesis. Specifically, we use computer simulation to estimate how close CR_{tot} and TEP are to their optimal values at the levels of TAN observed during hypertrophic remodeling and heart failure. From our simulations we are able to predict the impact of reducing CR_{tot} and TEP on maintaining the energetic state, compared to the control case where these pools are held fixed. We show that the hypothesis that CR_{tot} and TEP are maintained near levels that maintain physiological free energy of ATP hydrolysis is consistent with the experimentally observed levels of these metabolic pools.

II. METHODS

Simulations are based on a computer model simulating cardiac mitochondrial oxidative phosphorylation and tricarboxylic acid cycle as well as cytoplasmic ATP hydrolysis,

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creatine kinase reaction, and adenylate kinase reaction. The model has been parameterized and validated based on *in vitro* data obtained from purified mitochondria [10] and *in vivo* data on steady-state and transient phosphate metabolite in the heart under a variety of conditions and perturbations [9]. The components of the model are illustrated in Figure 1.

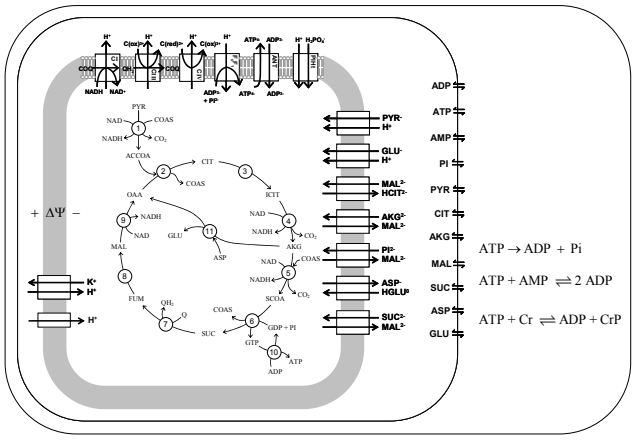

Fig. 1. Diagram of model used for simulations of cardiac energy metabolism. The internal compartment is the mitochondrial model of Wu et al. [10], [9]. Abbreviations used in this diagram and detailed description of the model may be found in Wu et al. [10], [9].

The reactions in the cellular compartment are ATP hydrolysis (ATP → ADP + Pi), adenylate kinase (ATP + AMP ⇌ 2 ADP), and creatine kinase (ATP + Cr ⇌ ADP + CrP). The mitochondrial compartment includes the reactions of the tricarboxylic acid cycle, the components of the respiratory chain, mitochondrial ATP synthase, and a number of metabolite transporters. The model takes the form of 57 ordinary differential equations, described in Wu et al. [11].

III. RESULTS

Figures 2, 3, 4, and 5 plot the model-predicted free energy of ATP hydrolysis, $\Delta G'_{ATP}$, as functions of CR_{tot} and TEP for the normal case (Figure 2) and in early (Figure 3), moderate (Figure 4), and severe (Figure 5) LVH. These predictions are obtained by setting TAN to the values associated with these stages of LVH, as indicated in the figures and varying CR_{tot} and TEP as indicated. All other model parameters are set to the normal physiological levels, as validated in Wu et al. [9].

All values of TAN, CR_{tot}, and TEP used to generate the relationships plotted above correspond to values obtained for the canine myocardium. The experimental LVH model is described in [11]. It is apparent from these simulations that in the diseased states CR_{tot} and TEP attain values for which $\Delta G'_{ATP}$ at the maximal work rate is nearly equal to that of the normal state. In fact, the analysis predicts that in moderate-stage LVH, the magnitude of $\Delta G'_{ATP}$ is actually increased by approximately 1 kJ·mol^{-1}. Thus it appears that the changing metabolic pool sizes may cause a *metabolic* or *energetic hypertrophy* that coincides with anatomical remodeling in early to moderate LVH. Given the expected range of experimental error associated with the

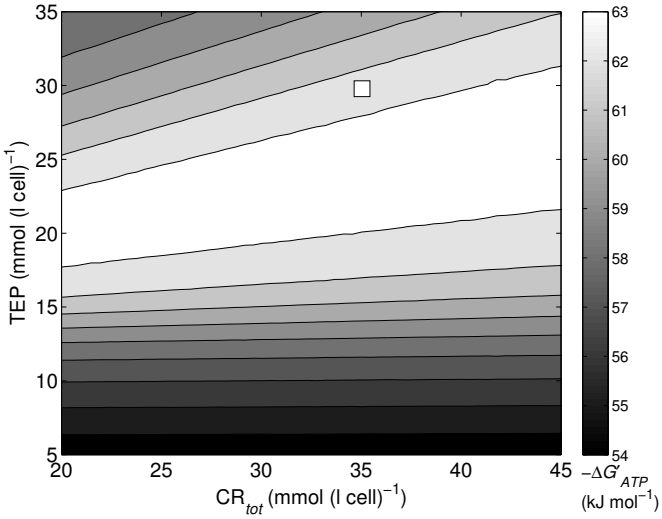

Fig. 2. Free energy of ATP hydrolysis at the maximal cardiac work rate for normal case. Here TAN is set to 8.62 mmol (l cell)$^{-1}$. The marked data point represents the physiologically normal values of CR_{tot} = 35.04 mmol·(l cell)$^{-1}$ and TEP = 29.78 mmol (l cell)$^{-1}$ [11].

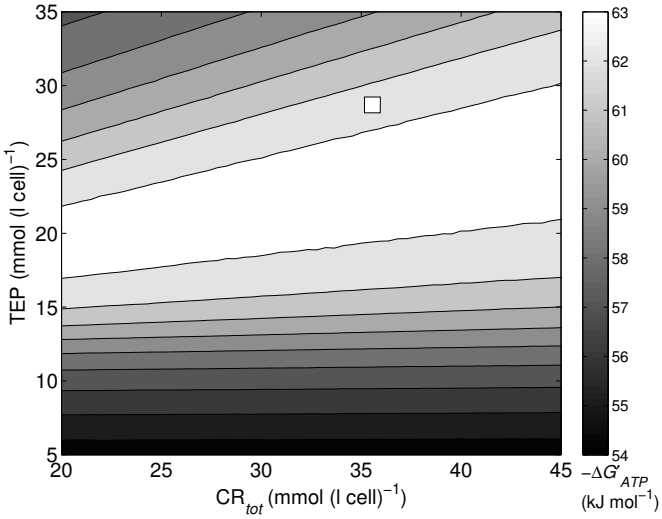

Fig. 3. Free energy of ATP hydrolysis at the maximal cardiac work rate for the early LVH case. Here TAN is set to 8.20 mmol (l cell)$^{-1}$. The marked data point represents the experimentally determined values that correspond to the above value of TAN observed in early-stage heart failure: CR_{tot} = 35.56 mmol (l cell)$^{-1}$ and TEP = 28.69 mmol·(l cell)$^{-1}$ [11].

metabolic pool estimates (approximately 5-10%) and uncertainty in model-based predictions, this predicted metabolic hypertrophy requires further experimental investigation.

Whether or not the magnitude of $\Delta G'_{ATP}$ increases during early and moderate LVH remodeling, it is at least apparent that CR_{tot} and TEP are maintained at or near values that allow the heart to maintain $\Delta G'_{ATP}$ (at the maximal work rate) at values that are nearer to the physiological level during hypertrophic remodeling and in heart failure than would be achieved by holding these pool sizes constant. We can explore this specific optimality hypothesis by comparing model predictions of $\Delta G'_{ATP}$ at the maximal cardiac work rate for different assumptions regarding the metabolic pools sizes,

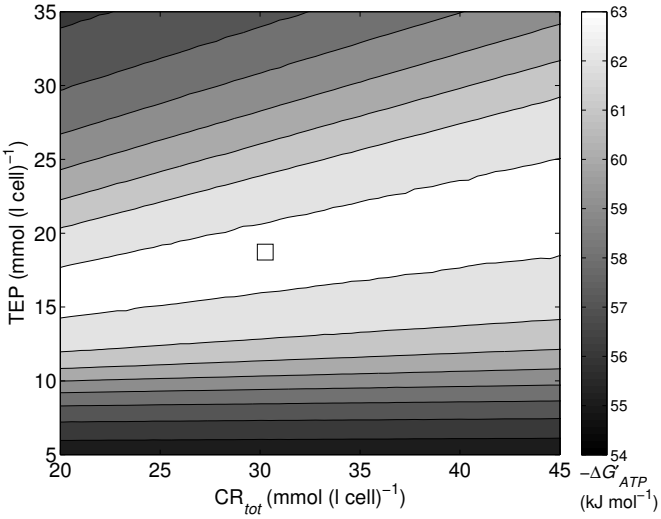

Fig. 4. Free energy of ATP hydrolysis at the maximal cardiac work rate for the moderate LVH case. Here TAN is set to 6.85 mmol (l cell)$^{-1}$. The marked data point represents the experimentally determined values that correspond to the above value of TAN observed in moderate-stage heart failure: CR$_{tot}$ = 30.26 mmol (l cell)$^{-1}$ and TEP = 18.72 mmol·l cell)$^{-1}$ [11].

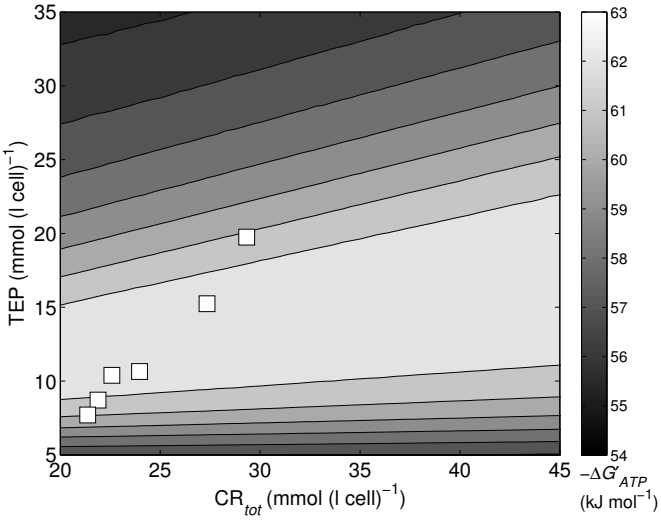

Fig. 5. Free energy of ATP hydrolysis at the maximal cardiac work rate for late LVH (failing heart) case. Here TAN is set to 3.52 mmol (l cell)$^{-1}$, corresponding to the average value determined from 6 canine hearts in end-stage heart failure reported in Wu et al. [11]. The marked data points represent CR$_{tot}$ and TEP values from 6 animals in end-stage heart failure.

as illustrated in Figure 6. Here we model the relationships between the metabolic pools and the ratio of left-ventricular weight to body weight (LVW/BW) as linear functions:

$$\begin{aligned} \text{TAN} &= -(0.5542)\cdot(\text{LVW/BW}) + (11.00), \\ \text{CR}_{tot} &= -(1.730)\cdot(\text{LVW/BW}) + (42.48), \\ \text{TEP} &= -(1.108)\cdot(\text{LVW/BW}) + (34.55), \end{aligned} \tag{1}$$

where the units of TAN, CR$_{tot}$, and TEP are mmol·(l cell)$^{-1}$ and (LVW/BW) is in units of gm·kg^{-1}. The slopes and intercepts of these relationships are obtained by matching the experimental data described above from hypertrophic remodeling (reported in [11].)

Figure 6 plots three curves representing $\Delta G'_{ATP}$ corresponding to a loss of TAN predicted by the above relationship. One curve (labeled "CR$_{tot}$ and TEP fixed") is computed with the CR$_{tot}$ and TEP held constant at the normal physiological values. The curve labeled "experimentally estimated" corresponds to model predictions made with CR$_{tot}$ and TEP varying according to estimated relationship between these pool sizes given above. The final curve (labeled "maximal") represents model predictions made with CR$_{tot}$ and TEP set to values that are associated with the maximal achievable value of $|\Delta G'_{ATP}|$.

From these simulations it is apparent that the model predicts that $|\Delta G'_{ATP}|$ steadily drops with LVW when TEP and CR$_{tot}$ are held constant and physiological levels. The free energy is predicted to increase (via the proposed *metabolic hypertrophy*) to match the maximal magnitude in moderate LVH. Beyond the range of moderate LVH (marked with a vertical dashed line), both the maximal and the experimentally estimated magnitude of $\Delta G'_{ATP}$ decrease. However, at all times the predicted value of $|\Delta G'_{ATP}|$ is greater than for the case where TEP and CR$_{tot}$ are held constant. Thus our model analysis predicts that, compared to the case where CR$_{tot}$ and TEP are held fixed, these pools are regulated such that the ATP hydrolysis potential is maintained at higher magnitude and closer to its normal physiological value.

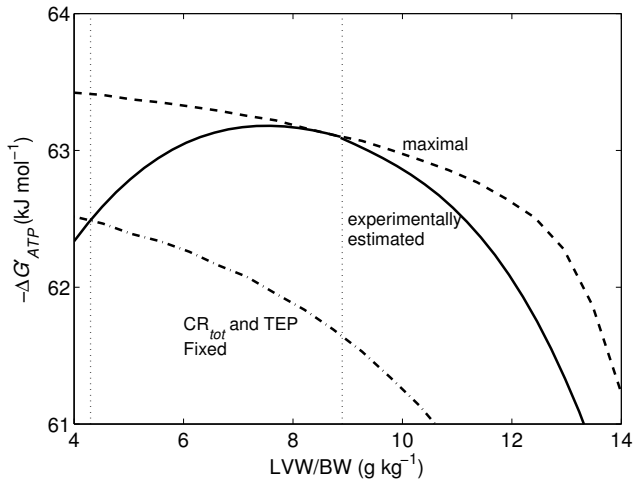

Fig. 6. Model-predicted $\Delta G'_{ATP}$ versus LVW/BW. The model-predicted $\Delta G'_{ATP}$ is plotted assuming TEP and CR$_{tot}$ attain values that predict maximal $|\Delta G'_{ATP}|$, assuming the experimentally estimated changes with hypertrophy, and assuming they are held fixed at normal physiological levels.

IV. CONCLUSIONS AND FUTURE WORKS

Our major conclusion is that during hypertrophic remodeling in the canine myocardium the pools of total creatine and total exchangeable phosphate change in such a way that, given the observed reductions in the total adenine nucleotide pool, the hydrolysis potential of the heart remains nearer to the normal physiological value than would be achieved

by holding these pools constant. Furthermore, during early and moderate LVH, our model analysis predicts that the energetic state of the heart (measured by the magnitude of $\Delta G'_{ATP}$ at the maximal cardiac work rate) increase—a form of metabolic hypertrophy.

The mechanisms that maintain this "fine balance" [8] of the TEP and CR_{tot} pools are not clearly understood. One possibly important mechanism of reduction of the adenine nucleotide pools is through loss of adenosine during ischemia/hypoxia [7]. Thus it is possible that loss of TAN (through adenosine) is a primary event compared to remodeling of CR_{tot} and TEP and that changes in CR_{tot} and TEP are regulated to maintain the energetic state of the heart in response to a given TAN level.

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