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## Altered Creatine Kinase Adenosine Triphosphate Kinetics in Failing Hypertrophied Human Myocardium

Craig S. Smith, MD; Paul A. Bottomley, PhD\*; Steven P. Schulman, MD; Gary Gerstenblith, MD; Robert G. Weiss, MD\*

**Background**—The progression of pressure-overload left ventricular hypertrophy (LVH) to chronic heart failure (CHF) may involve a relative deficit in energy supply and/or delivery.

**Methods and Results**—We measured myocardial creatine kinase (CK) metabolite concentrations and adenosine triphosphate (ATP) synthesis through CK, the primary energy reserve of the heart, to test the hypothesis that ATP flux through CK is impaired in patients with LVH and CHF. Myocardial ATP levels were normal, but creatine phosphate levels were 35% lower in LVH patients (n=10) than in normal subjects (n=14,  $P<0.006$ ). Left ventricular mass and CK metabolite levels in LVH were not different from those in patients with LVH and heart failure (LVH+CHF, n=10); however, the myocardial CK pseudo first-order rate constant was normal in LVH ( $0.36\pm 0.04\text{ s}^{-1}$  in LVH versus  $0.32\pm 0.06\text{ s}^{-1}$  in normal subjects) but halved in LVH+CHF ( $0.17\pm 0.06\text{ s}^{-1}$ ,  $P<0.001$ ). The net ATP flux through CK was significantly reduced by 30% in LVH ( $2.2\pm 0.7\text{ }\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$ ,  $P=0.011$ ) and by a dramatic 65% in LVH+CHF ( $1.1\pm 0.4\text{ }\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$ ,  $P<0.001$ ) compared with normal subjects ( $3.1\pm 0.8\text{ }\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$ ).

**Conclusions**—These first observations in human LVH demonstrate that it is not the relative or absolute CK metabolite pool sizes but rather the kinetics of ATP turnover through CK that distinguish failing from nonfailing hypertrophic hearts. Moreover, the deficit in ATP kinetics is similar in systolic and nonsystolic heart failure and is not related to the severity of hypertrophy but to the presence of CHF. Because CK temporally buffers ATP, these observations support the hypothesis that a deficit in myofibrillar energy delivery contributes to CHF pathophysiology in human LVH. (*Circulation*. 2006;114:1151-1158.)

**Key Words:** hypertrophy ■ adenosine triphosphate ■ heart failure ■ creatine kinase  
■ magnetic resonance spectroscopy ■ metabolism

Left ventricular hypertrophy (LVH) is, initially, an adaptive response to chronic pressure overload but is ultimately associated with a 10-fold greater likelihood of subsequent chronic heart failure (CHF).<sup>1,2</sup> Because pressure-overload LVH increases energetic cost, a mismatch in myocardial energy supply and demand may contribute to the development of heart failure in LVH. Myocardial energetic demands are met primarily through mitochondrial adenosine triphosphate (ATP) production via oxidative phosphorylation.<sup>3</sup> The creatine kinase (CK) reaction serves as the prime cardiac energy reserve, providing ATP during periods of increased demand by reversibly and rapidly converting adenosine diphosphate (ADP) and phosphocreatine (PCr) to ATP and creatine.<sup>4,5</sup> The PCr/ATP ratio is one indicator of the energetic state of the myocardium and is reduced in animal models of myocardial hypertrophy,<sup>6,7</sup> in human LVH,<sup>8-10</sup> and

in CHF.<sup>11,12</sup> Despite the potential importance of the cardiac PCr/ATP ratio for understanding cardiac energetics, this ratio does not directly reflect the rate of ATP production through the CK reaction,<sup>7,13-15</sup> which may be more important in the progression to CHF in patients with LVH.

### Clinical Perspective p 1158

In animal models of chronic pressure-overload LVH, <sup>31</sup>P magnetic resonance spectroscopy (MRS) magnetization transfer techniques demonstrate significant decreases in CK flux that are larger than the changes in the cardiac PCr/ATP ratio.<sup>7,13</sup> In both canine and swine models, the forward flux through CK is reduced by 30% to 50% in compensated LVH compared with normal hearts<sup>7,15</sup> and by 60% in those that developed CHF.<sup>7</sup> The abnormality in CK metabolism in those studies was related to the severity of hypertrophy.<sup>7</sup> However

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the degree of hypertrophy was greater in failing than nonfailing hearts,<sup>7,16</sup> and therefore, it was not possible to determine whether the reported metabolic changes were related to the degree of hypertrophy, the concomitant heart failure, or both. At least half of all patients with LVH-associated CHF have preserved systolic function,<sup>17</sup> a condition sometimes referred to as “diastolic heart failure.” The metabolic abnormalities associated with nonsystolic CHF have not been reported previously.

We recently developed a high-speed magnetization transfer technique that enabled, for the first time, direct measures of ATP flux through CK in the human heart.<sup>18</sup> A highly significant reduction in ATP synthesis from CK, even before any reduction in cardiac [ATP] can be detected, occurs in patients with nonischemic dilated cardiomyopathy (DCM) and mild-to-moderate CHF.<sup>18</sup> Reduced ATP buffering by CK in DCM could potentially contribute to the systolic dysfunction.<sup>18</sup> As yet, there are no reports of CK flux in the hypertrophied human heart, or indeed in any other condition that progresses to CHF. Because the underlying pathophysiology, signaling cascades, and energy demands of pressure-overload LVH differ from those of DCM, the extent to which ATP kinetics are altered in human LVH in the presence and absence of CHF is not known. We therefore used the same <sup>31</sup>P MRS 4-angle saturation transfer (FAST) magnetization transfer technique to directly measure myocardial ATP turnover through CK in patients with compensated and failing LVH, as defined by a modified Framingham scoring system,<sup>19</sup> to test the hypothesis that CK energy delivery is altered in human pressure-overload hypertrophy with or without associated CHF. The present study, is the first (to the best of our knowledge) to address whether changes in ATP kinetics in failing hypertrophied hearts are simply related to the degree of hypertrophy or are a characteristic of the progression to heart failure and whether they occur in both systolic and nonsystolic human heart failure.

## Methods

Additional details are provided in the online-only Data Supplement.

### Human Subjects

Studies were approved by The Johns Hopkins Institutional Review Board on human investigation, and all subjects gave informed consent. Fourteen healthy subjects (mean age 41±7 years; 2 women) without a history of hypertension, coronary artery disease, or diabetes mellitus were studied, including some previously reported who were contemporaneous with the current studies.<sup>18</sup> All subjects with LVH (n=20; age 56±12 years; 11 women) had stage II hypertension of at least 1-year duration with echocardiographic evidence of concentric hypertrophy (septal and posterior wall thickness >1.2 cm). All patients had either a cardiac catheterization within the prior 12 months that demonstrated no significant coronary stenosis or a negative stress test. In these 20 patients, 10 had CHF (LVH+CHF), as defined with a modified Framingham scoring system,<sup>19</sup> and 10 did not (LVH). CHF was diagnosed at the time of the patient's hospitalization with 2 major criteria (paroxysmal nocturnal dyspnea or orthopnea, jugular venous distention, rales, cardiomegaly, radiographic pulmonary edema, S<sub>3</sub> gallop, hepatogastric reflux, or >4.5-lb weight loss in response to diuresis) or 1 major and 2 minor criteria (ankle edema, night cough, dyspnea on exertion, hepatomegaly, or pleural effusion).<sup>19</sup> Because half of all patients with heart failure have preserved ejection fractions,<sup>17,20</sup> LVH+CHF patients were subdivided into 2 groups: those with

normal left ventricular systolic function (left ventricular ejection fraction ≥50% [“nonsystolic CHF”, n=5) and those with impaired left ventricular ejection fraction (left ventricular ejection fraction <50% [“systolic CHF”, n=5), as defined by prior ejection fraction criteria.<sup>17</sup> No magnetic resonance imaging/MRS studies were performed on patients during episodes of acute decompensated CHF.

### Cardiac MRS

Subjects were positioned prone in a clinical broadband 1.5T General Electric (Milwaukee, Wis) magnetic resonance imaging scanner on a <sup>31</sup>P MRS 6.5-cm receive, 20-cm transmit surface coil probe, and image-guided, 1-dimensional chemical shift imaging was used to spatially localize spectroscopic acquisitions (1-cm slice thickness) to the human heart. The FAST method was applied to measure ATP flux through the CK reaction with 2 pairs of <sup>31</sup>P acquisitions with adiabatic pulse flip angles of 15° and 60°: 1 pair with the  $\gamma$ -ATP resonance at -2.7 ppm saturated, and the other pair with the same irradiation applied at +2.7 ppm (control saturation).<sup>18,21</sup> A fifth <sup>31</sup>P MRS data set was acquired with a 60° pulse, without selective saturation, to measure both the spillover of the saturation and phosphate metabolite concentrations. A cardiac <sup>1</sup>H MRS data set was then acquired with a 60° pulse using the <sup>31</sup>P MRS receiver coil to provide a tissue water reference for the concentration measurements.<sup>22</sup> The image-guided spectroscopy examinations typically took a total of 60 to 75 minutes per subject.

[PCr] and [ATP] were calculated by 2 previously validated techniques that used water<sup>22</sup> and phosphate<sup>23,24</sup> as internal and external references, respectively, and the results were averaged for the myocardial slices in each subject.<sup>22-24</sup> The water-reference concentration measures ( $\mu\text{mol/g}$  tissue) assumed substantially equivalent tissue water content among the groups.<sup>22</sup> The pseudo first-order rate constant,  $K_{\text{for}}$  was calculated from the ratios of PCr signals in FAST data sets from equations 5, 6, and 9 of Bottomley et al,<sup>21</sup> corrected for spillover irradiation based on the unsaturated <sup>31</sup>P cardiac data.  $K_{\text{for}}$  is effectively the fraction of the PCr pool that exchanges with ATP each second. The CK forward flux rate was calculated from the product  $K_{\text{for}}[\text{PCr}]$  (in  $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{s}^{-1}$ ) for each subject.

### Statistical Analysis

Continuous variables are presented as mean±SD, whereas categorical variables are presented as either absolute counts or percentages. Statistical analysis of demographic variables was performed with an unpaired, 2-tailed Student *t* test. The Shapiro-Wilk test was used to test the normality of the key metabolic variables, and it could not reject the hypothesis that all these variables are normally distributed. Therefore, parametric testing was performed, and differences in means among groups were assessed by ANOVA followed by groupwise comparisons with the Tukey-Kramer adjustment to control the overall error rate. In all instances, statistical significance was assumed at  $P<0.05$ .

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

## Results

Baseline demographics are presented in Table 1. Subjects with LVH+CHF were significantly younger and had lower ejection fractions than those with LVH alone. Importantly, the degree of hypertrophy, as measured by the left ventricular mass index and by septal and posterior wall thickness, was the same in LVH and LVH+CHF patients.

Conventional transaxial cardiac <sup>1</sup>H images and corresponding myocardial 60° <sup>31</sup>P FAST spectra from a patient with LVH+CHF are shown in Figure 1. The PCr and ATP peak areas with control irradiation are proportional to the concentrations of the metabolites, spillover effects notwithstanding. The fractional reduction in the PCr peak with [ $\gamma$ ]-ATP

TABLE 1. Demographics

	LVH (n=10)	LVH+CHF (n=10)
Gender, female:male, n	6:4	5:5
Age, y	63±10	50±10*
Body surface area, m <sup>2</sup>	1.9±0.2	2.0±0.3
Heart rate, bpm	71±12	76±12
Blood pressure, mm Hg		
Systolic	143±18	145±25
Diastolic	80±16	80±10
Left ventricular mass index, g/m <sup>2</sup>	166±50	167±30
Ejection fraction, %	61±9	46±18*
Atrial fibrillation, %	0	10
Creatinine, mg/dL	0.94±0.3	1.32±0.5
Diabetes mellitus, %	50	50
History of tobacco use, %	50	70
Current tobacco use, %	30	40
Percentage with use of:		
β-Blocker	40	70
Angiotensin-converting enzyme inhibitor	60	30
Angiotensin receptor blocker	10	0
Loop diuretic	20	60
Spironolactone	0	10
Digoxin	0	0

Left ventricular mass index and ejection fraction were determined with echocardiography.

\* $P < 0.05$ .

saturated is proportional to the ATP flux through the CK reaction.

Metabolite concentrations,  $K_{for}$ , and CK flux data are summarized in Table 2. Myocardial [ATP] was not significantly reduced in LVH or LVH+CHF compared with that in normal subjects. However, mean cardiac [PCr] was reduced by 30% in both LVH and LVH+CHF compared with values observed in normal subjects (Table 2). Therefore, myocardial PCr/ATP was reduced by 30% in both LVH and LVH+CHF compared with normal (1.9±0.3 versus 1.3±0.4 in all 20 patients,  $P < 0.002$ ) but did not differ significantly between LVH groups with and without CHF (1.3±0.3 in LVH versus 1.3±0.5 in LVH+CHF,  $P = NS$ ).

TABLE 2. High-Energy Phosphate Concentrations and CK Flux

	Normal (n=14)	LVH (n=10)	LVH+CHF (n=10)
[PCr], μmol/g	9.4±1.1	6.1±2.0*	7.2±3.7
[ATP], μmol/g	5.5±1.3	4.7±1.3	5.0±1.1
PCr/ATP	1.9±0.3	1.3±0.3†	1.3±0.5†
$K_{for}$ , s <sup>-1</sup>	0.32±0.06	0.36±0.04	0.17±0.06‡
CK flux, μmol·g <sup>-1</sup> ·s <sup>-1</sup>	3.1±0.8	2.2±0.7§	1.1±0.4

\* $P < 0.006$  vs Normal, † $P < 0.003$  vs Normal.

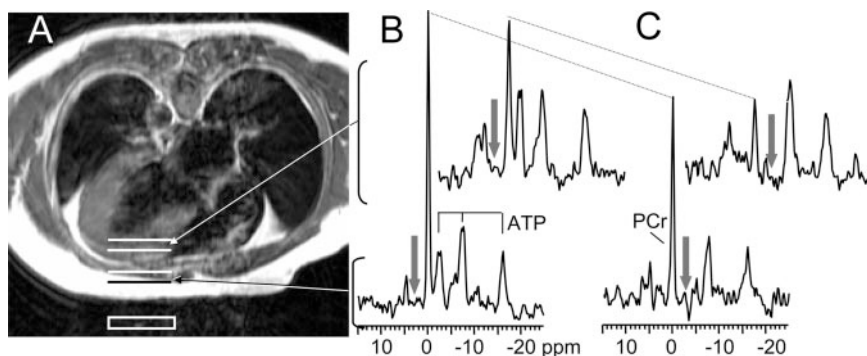
‡ $P < 0.001$  vs Normal and vs LVH.

§ $P = 0.011$  vs Normal.

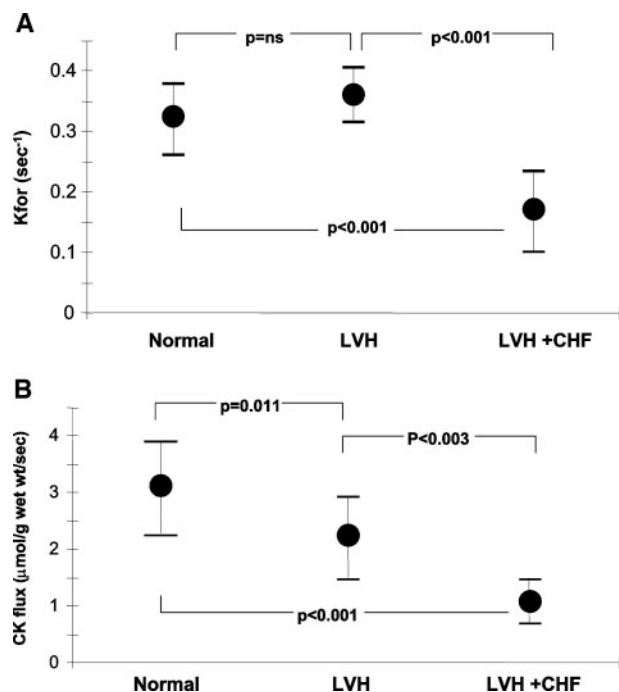
|| $P < 0.001$  vs Normal and  $P < 0.003$  vs LVH.

The kinetic data revealed more dramatic changes in the pseudo first-order rate constant and forward CK flux among the 3 groups than those seen in the absolute or relative pool sizes alone. The mean pseudo first-order rate constant,  $K_{for}$ , was similar in LVH and in normal subjects (Figure 2A). However, because of the reduction in [PCr], the net CK flux was reduced by 30% in the LVH group ( $P = 0.011$ ; Figure 2B). Moreover,  $K_{for}$  was reduced by 50% in LVH+CHF compared with normal subjects ( $P < 0.001$ ) and those with LVH alone ( $P < 0.001$ ). Mean CK flux in LVH+CHF was reduced by 65% compared with that in normal subjects ( $P < 0.001$ ; Table 2; Figure 2B) and by 30% compared with those with LVH ( $P < 0.003$ ).

Not only was LV mass similar in LVH and LVH+CHF (Table 1), but the abnormality in CK kinetics exhibited no significant correlation with the severity of hypertrophy, as measured by left ventricular mass index (Figure 3) and septal wall thickness ( $r^2 < 0.03$ , data not shown). Half of the patients with LVH+CHF (n=5) had nonsystolic heart failure with preserved LVEF (62±8%, range 55% to 75%), whereas the others (n=5) did not (30±7%, range 20% to 35%;  $P < 0.001$ ). LVH+CHF patients with nonsystolic heart failure and those with systolic heart failure had similar  $K_{for}$  (0.15±0.07 versus 0.18±0.06 s<sup>-1</sup>,  $P = NS$ ) and cardiac CK flux (1.02±0.07 versus 1.19±0.37 μmol · g<sup>-1</sup> · s<sup>-1</sup>, respectively,  $P = NS$ ). Accordingly,  $K_{for}$  and CK flux did not correlate with ejection fraction (Figure 3). Thus, in hypertrophic hearts, a low  $K_{for}$  appeared to be specific for the progression to heart failure and occurred without lower [ATP]. Highly significant reductions in the CK pseudo first-order rate constant and CK flux occur



**Figure 1.** Axial spin-echo MR image (A) of a patient with LVH+CHF lying prone over a <sup>31</sup>P surface coil (white box) and the corresponding localized <sup>31</sup>P MR spectra from the chest (lower 2 spectra) and left ventricle (upper 2 spectra). The resonances derive from PCr and the γ-, α-, and β-phosphate resonances of ATP. The spectra were acquired with a 60° flip angle in the presence of chemically selective saturating irradiation (arrows) either in the control (B) or γ-ATP position (C). The decrease in the height of the PCr peak between control and γ-ATP saturation (dotted lines) is directly related to the rate of ATP synthesis through the CK reaction.



**Figure 2.** A, CK myocardial pseudo first-order rate constant ( $K_{for}$ ) for healthy subjects (Normal), patients with LVH, and others with LVH with CHF (LVH+CHF). Note that  $K_{for}$  is not reduced by LVH but only in LVH+CHF. B, Net ATP flux through the CK reaction (CK flux) in healthy subjects (Normal), patients with LVH, and others with LVH with CHF (LVH+CHF).

in hypertrophied patients with both systolic and nonsystolic heart failure and are not directly related to the severity of LVH per se.

### Discussion

The present study is the first to report *in vivo* measures of ATP flux through the CK reaction, the prime energy reserve, in hypertrophic human hearts. We found that in the absence of CHF, ATP flux through CK is reduced by 30% in LVH patients, and this reduction can be accounted for by a proportionate loss of substrate (PCr). Patients with a similar degree of LVH but who additionally had CHF exhibited no greater reductions in PCr and ATP than those without CHF. However, the CK pseudo first-order rate constant was markedly depressed in these CHF patients compared with that of normal subjects and of LVH patients without CHF. Consequently, myocardial CK flux in LVH patients with CHF declined to approximately one third that of normal subjects. These data provide direct evidence that a defect in the kinetics of ATP turnover through CK and not the pool sizes per se may distinguish failing and nonfailing human hypertrophic myocardium.

### Myocardial High-Energy Phosphate Metabolism and the Role of CK

Myocardial ATP synthesis via oxidative phosphorylation is directly related to myocardial oxygen consumption, which is not decreased but is normal or increased at rest in animal models of pressure-overload LVH<sup>15,16</sup> and in patients with pressure-overload LVH.<sup>25</sup> In addition, detailed studies of

deoxyhemoglobin have failed to detect evidence of an oxygen deficit in LVH.<sup>26</sup> Thus, if there is a metabolic energy defect associated with LVH, it is not obviously related to mitochondrial ATP synthesis via oxidative phosphorylation but may lie elsewhere.

The CK reaction is important in energy metabolism because of its ability to rapidly buffer ATP and to transfer high-energy phosphates between sites of production and utilization. Specific cytosolic and mitochondrial isoforms of CK maintain high ATP/ADP ratios at the myofibrillar sites of utilization and low ATP/ADP ratios at the mitochondrial sites of production. The CK cytosolic isozyme shift to more “fetal” isoforms in LVH models<sup>7,14,15</sup> and in human LVH<sup>4</sup> is postulated to offer a thermodynamic advantage. CK isoform analysis could not be performed in the present noninvasive study of stable ambulatory patients with LVH because there was no clinical indication for the requisite cardiac biopsy. Recent studies in CK knockout mice, however, suggest similar kinetic characteristics among the CK isoforms.<sup>27</sup>

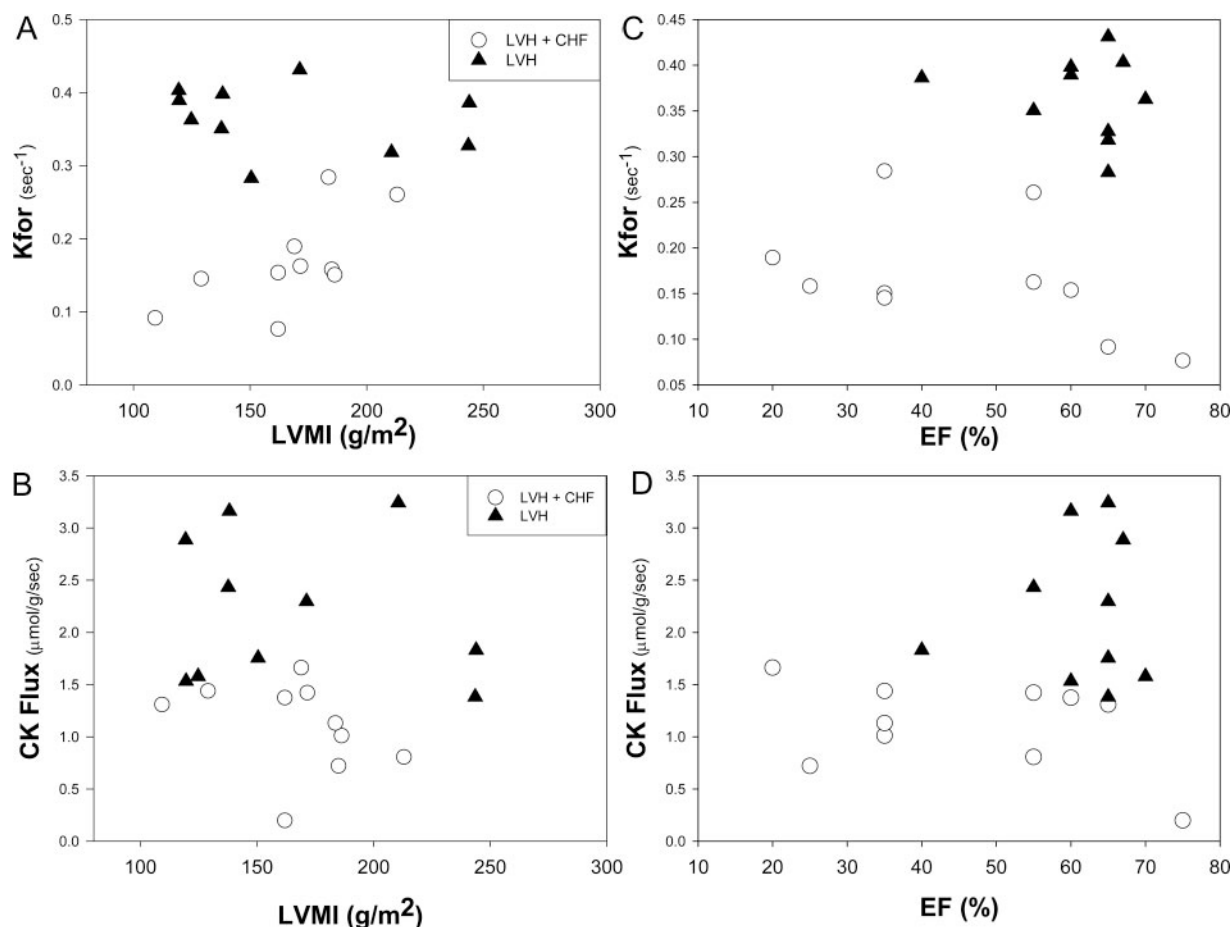
### CK Metabolite Levels in LVH

The myocardial PCr/ATP ratio has been used for more than 2 decades to index myocardial energy metabolism, and the reduction in PCr/ATP observed in LVH here is consistent with published results in animal models<sup>7,15</sup> and in patients with LVH and heart failure.<sup>8–10</sup> However, a reduced PCr/ATP is not specific to CHF, because patients with LVH but no heart failure can also have reduced PCr/ATP<sup>10,28</sup> (Table 2).

Measurements of absolute high-energy phosphate pools, rather than their ratios, have been proposed as a more discerning measure of energy metabolites.<sup>9,29,30</sup> However, the ATP pool was reported as nearly normal<sup>30</sup> or only modestly reduced<sup>4,9</sup> in compensated hypertrophy, and the current observations are consistent with those prior reports.

### ATP Turnover Through CK in LVH

The rate of turnover of high-energy phosphate pools may be more physiologically important than the relative or absolute size of the pools themselves. The similar relative and absolute metabolite pool sizes in LVH and LVH+CHF reveal the potential pitfalls in trying to infer CK metabolism from single measures of PCr/ATP or pool sizes alone, as is commonly done. The present findings are consistent with prior *in vitro*<sup>31</sup> and *in vivo*<sup>7,15</sup> CK flux measurements in pressure-overload animal models of LVH with <sup>31</sup>P nuclear magnetic resonance magnetization saturation techniques. In compensated canine and porcine LVH, the fraction of the PCr pool exchanging with ATP,  $K_{for}$ , was normal.<sup>7,15</sup> Because there was a 30% to 50% loss of [PCr], the forward CK flux was reduced by that amount in those settings, as observed in the present study in human LVH. In hypertrophied animal hearts that progressed to failure, there was a significant 35% decline in  $K_{for}$  that further reduced CK flux by 55% to 60% below normal.<sup>7</sup> Thus, the present data in human LVH and LVH+CHF are consistent with these prior animal studies in that we observed progressive decreases in CK flux for the compensated and failing hypertrophied myocardium but found reduced  $K_{for}$  only in failing myocardium (Table 2).



**Figure 3.** LVH severity, as indexed by left ventricular mass index (LVMI; A and B) and left ventricular ejection fraction (EF; C and D) versus ATP kinetics, as indexed by  $K_{\text{for}}$  (above) and CK flux (below) for LVH ( $\blacktriangle$ ) and LVH+CHF ( $\circ$ ). Neither  $K_{\text{for}}$  nor CK flux correlated with the severity of LVH ( $r^2 < 0.03$  for both in all 20 patients) or with ejection fraction. It is the presence of CHF, not the severity of LVH, that is associated with reduced ATP synthesis through CK. Reduced CK flux occurs in failing hearts with impaired and preserved ejection fractions.

Additionally, our observations allow new insights not available from prior studies in animals. First, these are original kinetic observations in human LVH. Second, hypertrophy was more severe in failing hearts than in nonfailing hearts in the animal models,<sup>7,15</sup> and it was concluded that the reduction in energetics was related to the severity of hypertrophy. Unlike animal models, in which the stimulus is abrupt, human pressure-overload LVH due to hypertension often develops over a longer time period and in the presence of antihypertensive therapies. This clinical setting for the first time provided the ability to determine the effect of heart failure on CK kinetics in LVH, because patients with and without CHF had similar degrees of hypertrophy (Table 1). Thus, although LVH results in a loss of PCr (Table 2), reduced kinetics is closely tied to the presence of heart failure and not to the severity of LVH (Figure 3).

These observations in LVH also provide novel insights and extend results from those previously reported for human DCM.<sup>18</sup> First, reductions in PCr/ATP and [PCr] are greater here in LVH and LVH+CHF (30%) than in DCM (10% to 20%) by these methods.<sup>11,18</sup> Second, the relative reduction in cardiac CK flux from normal values was greater in the present study in LVH+CHF (65%) than in DCM (50%).<sup>18</sup> Indeed, the

failing hypertrophic hearts studied here have the greatest deficits in CK flux yet studied in humans. Third, these data provide the first information on CK energy turnover in diastolic heart failure with preserved systolic function and demonstrate that reduced CK flux is common to both systolic and nonsystolic human heart failure.

### Mechanisms Contributing to Altered CK Kinetics in LVH+CHF

In general, reductions in CK flux may be due to a loss of total enzyme activity ( $V_{\text{max}}$ ), altered substrate ratios, or allosteric modification of the enzyme. Because PCr is a substrate for the forward CK reaction, reduced [PCr] lowers CK flux in human LVH without CHF. However, reduced [PCr] cannot account for the further decline in CK flux observed in LVH+CHF, because PCr is not further depleted with CHF (Table 2). The larger flux reduction in human CHF arises from a decrease in  $K_{\text{for}}$ . Total in vitro CK enzyme activity is preserved in LVH models without heart failure<sup>7,15</sup> but is reduced in those with heart failure<sup>7</sup> and in LVH patients at the time of cardiac surgery.<sup>4</sup> These observations support the view that the decrease in  $K_{\text{for}}$  observed in the present study in hypertrophic hearts that failed was at least in part due to a loss

of CK enzyme. Total myocardial creatine content is 40% to 50% lower in patients with LVH+CHF than in asymptomatic LVH patients,<sup>32</sup> despite the similar PCr content (Table 2). Taken together, the CK equilibrium reaction<sup>33</sup> predicts lower [ADP] in LVH+CHF than in LVH. This, as well as less CK enzyme ( $V_{max}$ ), likely contributes to the lower  $K_{for}$  and CK flux in LVH+CHF hearts.<sup>33</sup>

### Consequences of Altered CK Kinetics in LVH+CHF

Because cardiac CK flux is important for temporal ATP buffering, it appears unlikely that a significant reduction in CK flux would be adaptive or protective in LVH or LVH+CHF. Reducing CK flux in normal animal hearts by PCr depletion impairs contractile function<sup>34</sup> and eliminates survival after infarction.<sup>35</sup> Genetic CK knockout reduces *in vivo* contractile reserve,<sup>36</sup> and knockout of both the M- and mito-isoforms of CK also limits contractile recovery after ischemia.<sup>37</sup> However, subcellular reorganization<sup>38</sup> and increased activity of other ATP buffers and phosphotransfer networks,<sup>39</sup> such as adenyl kinase, occur after CK knockout and serve to attenuate the consequences of CK loss in those settings. Other recent work demonstrates that PCr depletion, and hence reduced CK capacity, adversely affects actinomyosin function even when [ATP] is normal.<sup>40</sup>

Would a reduction in CK flux of the magnitude observed in LVH+CHF be sufficient to limit mechanical function in the hypertrophied heart? Because CK is important for buffering cardiac ATP over time, one can calculate the impact of a 65% to 70% loss of CK buffering capacity. In the steady state, ATP synthesis rates must match those of ATP utilization to keep [ATP] constant during the cardiac cycle.<sup>41–43</sup> The mean basal rate of ATP production via oxidative phosphorylation is  $\approx 0.4 \mu\text{mol} \cdot \text{g}_{\text{ww}}^{-1} \cdot \text{s}^{-1}$  (where  $\text{g}_{\text{ww}}$  indicates gram of wet weight) in the normal human heart.<sup>44,45</sup> Because roughly 75% of ATP utilization occurs by peak force generation in each cardiac cycle,<sup>46,47</sup> one can conservatively anticipate that 75% of ATP utilization occurs during the first 150 ms of each contraction in the normal heart. The difference between this early ATP requirement ( $0.75 \times 0.4$  to  $\approx 0.30 \mu\text{mol}/\text{g}_{\text{ww}}$ , assuming a heart rate of 60 bpm) and the amount provided by *de novo* ATP synthesis during the same time period ( $0.4 \mu\text{mol} \cdot \text{g}_{\text{ww}}^{-1} \cdot \text{s}^{-1} \times 0.15 \text{ s}$  to  $\approx 0.06 \mu\text{mol}/\text{g}_{\text{ww}}$ ) must be met by temporal ATP buffers, of which CK is primary. To provide the requisite ATP ( $0.24 \mu\text{mol}/\text{g}_{\text{ww}}$ ) in 0.15 second, a CK flux rate of at least at  $1.6 \mu\text{mol} \cdot \text{g}_{\text{ww}}^{-1} \cdot \text{s}^{-1}$  is required. The observation that most patients with LVH and no CHF symptoms have mean CK flux ( $2.2 \pm 0.7 \mu\text{mol} \cdot \text{g}_{\text{ww}}^{-1} \cdot \text{s}^{-1}$ ) above this level and those with CHF have CK flux ( $1.1 \pm 0.4 \mu\text{mol} \cdot \text{g}_{\text{ww}}^{-1} \cdot \text{s}^{-1}$ ) at or below this level is therefore consistent with a hypothesis that the reduction in CK flux in LVH+CHF is of sufficient magnitude to compromise the ATP buffering capacity over time and thereby limit ATP availability to the myofibrils. This mechanism would impact both systolic and diastolic function. Because ATP demand is higher in pressure-overload hypertrophied hearts than in normal hearts, temporal buffering by CK may be even more important and the consequences of reduced CK flux more limiting in LVH

than in the normal hearts used in these calculations. Note that this mechanism would also mean that the hypertrophied heart is more metabolically susceptible to stressors such as increased chronotropic and inotropic demand and ischemia. Glucose and glycogen provide another rapid source of ATP in many tissues, including muscle.<sup>48</sup> In this regard, the “substrate switch” toward increased reliance on glucose observed in some heart failure models and strategies to increase glucose utilization in hypertrophic hearts<sup>49</sup> may also act as an additional rapid source of ATP during the cardiac cycle when the CK reservoir falls in CHF.

The present observations are consistent with but do not prove that inadequate ATP delivery via the CK reaction contributes to heart failure in patients with pressure-overload LVH. Unfortunately, a means to increase CK activity in the failing human heart has yet to be identified, and thus, the contractile consequences of such a metabolic intervention are unknown. Many genetic and phenotypic factors, including neuroendocrine, intracellular signaling cascades, extracellular changes, and mechanical remodeling, have all been shown to be associated with the progression to failure in hypertrophic hearts.<sup>50,51</sup> The present metabolic findings do not diminish the importance of these factors but rather offer the added perspective that reduced ATP supply may act as both an initiating event and a consequence. Inadequate ATP availability would initiate and accentuate the adverse consequences of energy-dependent pathways.<sup>50</sup> Conversely, factors that increase energy demand, such as adrenergic stimulation and biomechanical remodeling, exaggerate any energetic deficit.<sup>51</sup> Future studies to define the interactions between inadequate energy delivery and well-characterized pathways that contribute to the pathophysiology of LVH and CHF are needed.

### Conclusions

These results demonstrate that it is the kinetics of CK energy rather than the metabolite pool sizes or relative ratios that distinguishes compensated and failing hypertrophic human hearts. The findings are consistent with prior animal studies and demonstrate for the first time that reduced ATP kinetics is related to the development of heart failure rather than to the severity of hypertrophy. The reduction in CK flux in LVH+CHF is greater than that previously reported in human DCM, is similar in systolic and nonsystolic heart failure, and may be of a sufficient magnitude to impair the temporal buffering of ATP at the myofibrils. This provides a potential metabolic mechanism contributing to the systolic and diastolic dysfunction in failing human hypertrophied myocardium and provides a rationale to guide new investigations into means of augmenting CK flux in hypertrophied failing hearts.

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### CLINICAL PERSPECTIVE

Pressure-overload left ventricular hypertrophy (LVH) is associated with an increased risk of chronic heart failure (CHF). Because metabolism is required to fuel mechanical function and because myocardial energy demands are increased in pressure-overload LVH, a relative deficit in energy supply and/or delivery may accompany the development of CHF in patients with pressure-overload LVH. We used a new magnetic resonance technique to directly measure the rates of myocardial adenosine triphosphate (ATP) flux through creatine kinase (CK), the major energy reserve of the heart, for the first time in hypertensive patients with LVH alone and in others with LVH and CHF. We report that it is not the relative or absolute CK metabolite pool sizes but rather the kinetics of ATP turnover through CK that distinguish failing from nonfailing hypertrophic human hearts. Moreover, the deficit in ATP kinetics is similar in systolic and nonsystolic heart failure and is not related to the severity of hypertrophy but to the presence of CHF. These observations are consistent with a hypothesis that a deficit in myofibrillar energy delivery contributes to CHF pathophysiology in human LVH. The findings suggest that pharmacological agents that reduce energetic demand may be of particular benefit in pressure-overload LVH and that new strategies to augment CK-supported energy metabolism should be evaluated.