Creatine Kinase Over-Expression Improves Myocardial Energetics and Function in Failing Mouse Hearts

M. Y. Maslov[1], V. P. Chacko[2], M. Stuber[2], A. L. Moens[1], D. A. Kass[1], H. C. Champion[1], and R. G. Weiss[1,2]

[1]Department of Medicine, Cardiology Division, The Johns Hopkins University, School of Medicine, Baltimore, MD, United States, [2]Department of Radiology, Division of Magnetic Resonance Research, The Johns Hopkins University, School of Medicine, Baltimore, MD, United States

Introduction: Biochemical energy is absolutely required for normal cardiac contractile function and an energy deficit has been postulated to contribute to the contractile dysfunction in HF. Cardiac energetics are often indexed by the phosphocreatine (PCr) to ATP ratio (PCr/ATP), and a reduced PCr/ATP has been reported in both left ventricular hypertrophy (LVH) due to chronic pressure-overload (1) and in HF (2). The creatine kinase (CK) system is the prime energy reserve of the heart reversibly interconverting PCr and ATP and serving as an energy buffer matching cyclic ATP demands (3). Reduced CK expression and activity also occur in LVH and HF. Because conventional interventions have failed to improve PCr/ATP or CK activity in failing hearts, it has not been clear whether CK abnormalities are a consequence of HF or actually contribute to the mechanical dysfunction. In this study, a recently developed viral vector with the gene encoding for the CK-B isoform (4) was combined with *in vivo* cardiac MRS/MRI techniques in mice to address this long-standing issue.

Purpose: To transfect LVH murine hearts with either adeno-CK-B or a marker gene (Ad-β-gal) and then use *in vivo* MRS/MRI to determine whether augmented CK expression improves cardiac energy metabolism and whether that in turn enahances LV function and limits remodeling in HF.

Methods: LVH was induced by chronic pressure overload following permanent thoracic aorta constriction (TAC). Two weeks after TAC, mice underwent intra-aortic vector delivery (5) with either adenovirus encoding reporter β-galactosidase (TAC+Ad-β-gal, n=5) or CK-B (TAC+Ad-CK-B, n=9). Other mice that did not undergo TAC or transduction served as normal controls (n=9). One week after transduction, *viz.,* 3 weeks after TAC, *in vivo* MRI/MRS was performed on a Bruker Biospec NMR/MRI spectrometer equipped with a 4.7T/40 cm Oxford magnet and a BGA12 gradient set (Bruker Biospin MRI, Billerica). Cine MR images were acquired with a modified FLASH sequence (15 frames, TE=1.8 msec, TR=7 msec, flip angle = 30°) and spacially localized cardiac ^{31}P NMR spectra were measured with one-dimensional chemical shift imaging, using modified adiabatic pulses, in a custom built dual coil RF probe; and data analyses were performed as previously described (6,7).

Results: As compared with normal mice, TAC+Ad-β-gal mice developed pronounced LVH and had a 40% reduction in cardiac PCr/ATP (2.0±0.1 *vs* 1.2±0.1, normal *vs* TAC+Ad-β-gal, mean±SD, p<0.0007, see Figure 1A and 1B). LV remodeling occurred with increased LV mass (74±4 mg *vs* 170±33 mg, normal *vs* TAC+Ad-β-gal; p<0.003) and end diastolic volume (EDV, 48±3 μL *vs* 62±13 μL, normal *vs* TAC+Ad-β-gal; p<0.05) along with mechanical dysfunction as indexed by increased end systolic volume (ESV) and lower ejection fraction (EF) (Fig. 2).

In separate animals, Ad-CK-B transduction resulted in several fold increase in CK-B expression and in increased total CK activity by 40%. Ad-CK-B transduction significantly improved the *in vivo* cardiac PCr/ATP (1.2±0.1 *vs* 1.8±0.2, TAC+Ad-β-gal *vs* TAC+Ad-CK-B, p<0.0007). Ad-CK-B transduction also limited LV remodeling with lower LV mass and higher systolic function with increased EF and lower ESV (Fig. 2). EF correlated with PCr/ATP (r=0.75, p<.05 for n=14)

Conclusions: In a mouse model of pressure-overload TAC resulting in reduced PCr/ATP, significant remodeling, and LV contractile dysfunction similar to those reported before in human HF, CK-B over-expression can be achieved by Ad-CK-B transduction leading towards normalized cardiac PCr/ATP. In addition, LV remodeling and contractile function are significantly improved and the extent of functional improvement correlates with *in vivo* energetics. These data demonstrate for the first time that augmenting impaired CK energetics in HF limits its progression and improves cardiac function, strongly suggesting that the failing heart, as it relates to CK, is energy "starved".

References:

1.Bache RJ, Zhang J, et al. Cardiovasc Res. 1999;42(3):616-26.
2. Ingwall JS. Circulation 87: VII-58-VII-62, 1993
3. Ingwall JS, et al. N Engl J Med. 1985;313(17):1050-4
4. Auricchio A, et al PNAS 2001; 98, 5205-5210.
5. Champion HC, et al. Circulation. 2003 Dec 2;108(22):2790-7.
6. Naumova AV, et al Am J Physiol Heart Circ Physiol. 2006 Feb;290(2):H837-43.
7. Maslov M, et al Am J Physiol Heart Circ Physiol. 2006 Sep 8; [Epub ahead of print]

Acknowledgments: HL63030, HL61912 and the David E. Gibbons Award from the Mid-Atlantic Affiliate of the AHA

Figure 1A. Typical transverse short-axis ^{1}H MRI of a mouse thorax through mid LV. This is a frame at end diastole from the cine movie.

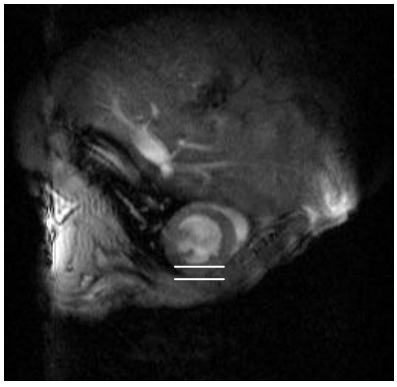

Figure 1B ^{31}P spectrum from the anterior cardiac slice is shown with the prominent peaks of phosphocreatine (PCr) and beta-phosphate of ATP ([β-P]ATP)

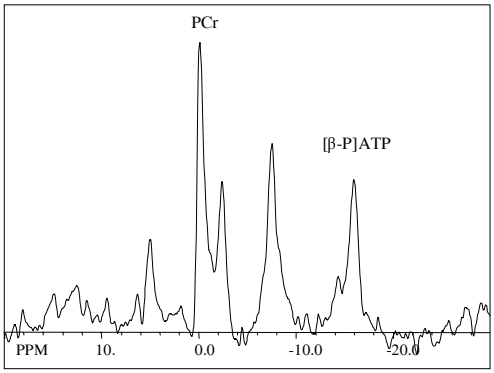

Figure 2. LV morphology and function;
* - p≤ 0.05 compared to control mice
+ - p≤ 0.05 compared to 3 week TAC+Ad-β-gal mice