

# Local energetic regulation of sarcoplasmic and myosin ATPase is differently impaired in rats with heart failure.

Frederic Joubert, James R. Wilding, Dominique Fortin, Valérie Domergue-Dupont, Marta Novotova, Renée Ventura-Clapier, Vladimir Veksler

▶ **To cite this version:**

Frederic Joubert, James R. Wilding, Dominique Fortin, Valérie Domergue-Dupont, Marta Novotova, et al.. Local energetic regulation of sarcoplasmic and myosin ATPase is differently impaired in rats with heart failure.: Local energy regulation is impaired in HF rats. The Journal of Physiology, 2008, 586 (Pt 21), pp.5181-92. 10.1113/jphysiol.2008.157677 . inserm-00325088

**HAL Id: inserm-00325088**

**https://inserm.hal.science/inserm-00325088**

Submitted on 19 Feb 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Local Energetic Regulation of Sarcoplasmic and Myosin ATPase is Differently Impaired in Rats with Heart Failure

**Running title: Local energy regulation is impaired in HF rats**

*Frederic Joubert*[1,2], *James R. Wilding*[1,2], *Dominique Fortin*[1,2], *Valérie Domergue-Dupont*[2], *Marta Novotova*[3], *Renée Ventura-Clapier*[1,2], *Vladimir Veksler*[1,2]

[1]INSERM, UMR-S 769, F-92296 Châtenay-Malabry, France;

[2]Univ Paris-Sud 11, IFR141, F-92296 Châtenay-Malabry, France;

[3]Institute of Molecular Physiology & Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic

Address for correspondence: Dr Frédéric Joubert
UMR-S 769 INSERM
Faculté de Pharmacie, Université Paris-Sud
5 rue J-B Clément, 92296 Châtenay-Malabry
France

Tel.: (33-1) 46.83.57.17.

Fax: (33-1) 46.83.54.75.

E-mail: frederic.joubert@u-psud.fr

Key words: Muscle energetics, heart failure, compartmentation

Number of words: 5632

**Abstract**

Local control of ATP/ADP ratio is essential for efficient functioning of cellular ATPases. Since creatine kinase (CK) activity and mitochondrial content are reduced in heart failure (HF), and cardiomyocyte ultrastructure is altered, we hypothesized that these changes may affect the local energetic control of two major cardiac ATPases, the sarcoplasmic reticulum (SR) Ca-ATPase (SERCA) and the myosin ATPase. Heart failure was induced by aortic stenosis in rats. Electron microscopy confirmed that failing cardiomyocytes had intracellular disorganization, with fewer contacts between mitochondria and myofibrils. Despite normal SERCA protein content, spontaneous $Ca^{2+}$ release measurements using Fluo-4 on saponin-permeabilized cardiomyocytes, showed a lower SR loading in HF even when endogenous CK and mitochondria were fully activated. Similarly, in permeabilized fibers, SR $Ca^{2+}$ loading supported by SR-bound CK and mitochondria was significantly reduced in HF (by 49% and 40% respectively, 43% when both systems were activated, $p<0.05$). Alkaline phosphatase treatment had no effect, but glycolytic substrates normalized calcium loading in HF to the sham level. The control by CK and mitochondria of the local ATP/ADP ratio close to the myosin ATPase (estimated by rigor tension) was also significantly impaired in HF fibers (by 32% and 46%, respectively). However, while the contribution of mitochondria and CK to local ATP regeneration were equally depressed in HF for the control of SERCA, mitochondrial contribution was more severely impaired than CK ($p<0.05$) with respect to myofilament regulation. These data show that local energetic regulation of essential ATPases is severely impaired in heart failure, and undergoes a complex remodeling as a result of a decreased activity of the ATP-generating systems and cytoarchitecture disorganization.

Key words: heart failure, mitochondria, creatine kinase, energetic regulation, compartmentation, remodeling

## Introduction

Calcium cycling defects greatly contribute to cardiomyocyte dysfunction in heart failure (HF). A reduced calcium transient is typically observed in heart failure, and results from a reduced amount of calcium stored in the sarcoplasmic reticulum (SR). This is classically thought to be due to a reduced SR-ATPase (SERCA) function and/or enhanced calcium leak through ryanodine receptors (RyR)(Marx et al., 2000; Bers, 2006; Belevych et al., 2007) .

However, a number of additional factors may be involved in cardiac dysfunction, such as dysfunction of the contractile machinery, cytoarchitectural disorganization and energetic starvation. Data from several laboratories, including our own (Garnier et al., 2003; Ingwall & Weiss, 2004; Ventura-Clapier et al., 2004) indicate that "energy deficiency" occurs in, and contributes to many pathophysiological processes. Heart muscle is among the highest ATP consumer organs. It has the highest content of mitochondria and an efficient system of energy transfer kinases which ensure that energy consumption by ATPases is exactly matched by energy production. Failing hearts have respective reductions in the activity and content of creatine kinase and mitochondria, which correlate with reduced working capacity (De Sousa et al., 1999).(Dzeja et al., 2000; Ventura-Clapier et al., 2004; Ingwall & Weiss, 2004) Among other pathological processes, the engagement of the SR Ca-ATPase pump in a futile cycle to maintain SR Ca load against a large ryanodine receptor-mediated leak in HF, is costly in terms of ATP consumption (Bers, 2006). Energy deficiency may therefore play a significant role in the pathogenesis of heart failure. Indeed, changes in energy charge (PCr/ATP ratio), which occur in the failing myocardium, represent a more accurate mortality prognosis than NYHA class or ejection fraction (Neubauer et al., 1997).

The critical match between energy supply and consumption does not take place in a bulk phase. The adult cardiomyocyte is a highly-organized cell with a crowded cytoplasm mainly composed of metabolically active and structured organelles that create microdomains at their boundaries with restricted diffusion and direct channeling of substrates to enzymes (Ventura-Clapier et al., 1998).(Saks et al., 2006; Weiss et al., 2006) The efficient functioning of myofibrils and sarcoplasmic reticulum (SR), necessary for a proper contraction and relaxation, strongly depends on ATP supply and ADP withdrawal (high localized ATP/ADP ratio) in the vicinity of myofibrillar and SR ATPases (Dzeja & Terzic, 2003). Indeed, local ADP accumulation considerably inhibits ATPase activity. We and others have shown that in cardiomyocytes, this is achieved by phosphotransfer kinases and mitochondria much more efficiently than by simple diffusion of nucleotides through the bulk cytosol. Creatine kinase

(CK) is bound to myofibrils and SR, and efficiently transfers the high-energy phosphate group of phosphocreatine (PCr) to ADP in the vicinity of the ATPases (Dzeja & Terzic, 2003). Mitochondria can themselves directly support myofibrillar/SR-calcium ATPase function as efficiently as the bound CK system, and much better than cytosolic ATP alone (Kaasik et al., 2001).(Saks et al., 2001) This indicates that a localized crosstalk between energy producing and energy consuming sites in the cardiac cell is required to ensure proper cardiac contractility. Local regulation and micro-compartmentation is not unique to energy metabolism but is rather a general property for optimal regulation of the cell, because the same holds true for many processes, including calcium regulation between mitochondria and SR, subsarcolemmal macromolecular complexes, or second messenger signaling. Cytoskeleton structurally participates in these local regulations, because in models of cytoarchitectural disorganization, such as the muscle LIM protein (MLP) or the desmin knockout mice, mitochondria-SR direct energy transfer is impaired (Wilding et al., 2006). Cytoskeletal alterations participate in cellular remodeling and in the alteration of mechanical function in the failing heart (Hein et al., 2000), and probably can also affect these local processes.

Because both CK activity and mitochondrial content are reduced in HF (De Sousa et al., 1999); (Ventura-Clapier et al., 2004; Ingwall & Weiss, 2004) and because the failing myocardium is known to exhibit cytoarchitectural disorganization that may compromise mitochondria, SR and myofilament interactions, we hypothesized that the localized control of ATPase activity by the local ATP/ADP ratio would be impaired and that this might contribute to contractile failure. Therefore, we examined the local regulation of ATPases by CK and mitochondria in situ in an animal model of heart failure. Since the energetic compartments controlling cellular ATPases are highly dependent on the intracellular architecture, our study was performed in saponin-permeabilized cells and fibers in which the cytoarchitecture is preserved. Our data reveal a striking relationship between function, energetics and cytoarchitecture within myocardium that participates in the contractile dysfunction of the cardiomyocyte.

**Methods**

All experiments performed conform to the European Community guiding principles in the care and use of animals (86/609/CEE, CE Off J no. L358, 18 December 1986), the local ethics committee (CREEA Ile-de-France Sud) guidelines, and the French decree no. 87-848 of October 19, 1987 (J Off République Française, 20 October 1987, pp 12245–12248).

Authorizations to perform animal experiments according to this decree were obtained from the French Ministère de l'Agriculture, de la Pêche et de l'Alimentation (no. 92-283, June 27, 2007). Briefly, aortic stenosis was mimicked in weaned male rats (60 to 70g) by placing a stainless steel hemoclip of 0.6mm ID on the ascending aorta via a thoracic incision, as previously described (De Sousa *et al.*, 1999). Control rats underwent the same procedure but without placement of the clip (sham operation). Animals were randomly assigned to one of two groups: sham-operated (sham, n=9) or heart failure (HF, n=9). Five months after surgery, rats were anesthetized by intraperitoneal injection of sodium thiopental (150mg/kg). Hearts were excised and rinsed in ice-cold calcium-free Krebs solution equilibrated with 95% $O_2$/5% $CO_2$ for 10 minutes. Hearts were dissected, with part of the tissue used for mechanical experiment, and part used for electron microscopy or biochemical experiments.

***Electron microscopy***

Hearts from five sham and five failing rats were excised and rinsed in ice-cold calcium-free Krebs solution for 10 minutes and fixed with 2% glutaraldehyde in cacodylate buffer for 2 hours as described previously (Wilding *et al.*, 2006). The papillary muscles were dissected and fixed in 2% glutaraldehyde in cacodylate buffer for 45 minutes and postfixed by 1% osmium tetroxide in cacodylate buffer for 30 min at room temperature. Tissue was stained with 1% aqueous solution of uranyl acetate, dehydrated in graded ethanol series, and embedded into Durcupan (ACM Fluka). Three tissue blocks of papillary muscle from each animal were obtained. Longitudinal sections were cut from each tissue block at three randomly selected levels separated by more than 50 µm. Ultrathin longitudinal sections (70 – 75 nm thick) were cut on a RMC PowerTome Ultramicrotome (Boeckeler Instruments, Inc., AZ, USA), placed on copper grids covered with formvar, stained with lead citrate and studied using JEM 1200 (JEOL, Japan) electron microscope. Images were recorded using a Gatan Dual Vision 300W CCD camera (Gatan Inc., USA).

***Myofibrillar intrinsic properties and estimation of SR calcium uptake***

Fibers (~200 µm width) were dissected from papillary muscles and permeabilized for 30 minutes in saponin (50 µg/ml), which preserves mitochondrial and SR function as described previously (De Sousa *et al.*, 1999); (Kaasik *et al.*, 2001; Wilding *et al.*, 2006) Fibers were mounted on a stainless-steel hook with a force transducer (AE 801, Microelectronics, Horton, Norway) as described previously (Kaasik *et al.*, 2001). Fibers were immersed in 2.5 ml chambers arranged around a disc and placed into a temperature-controlled bath and

positioned on a magnetic stirrer at 22°C. Sarcomere length was measured by laser diffraction and adjusted to 2.1 to 2.2 µm which gives maximum twitch. SR calcium loading capacity was measured in sham and HF rat cardiac fibers as described previously (Minajeva *et al.*, 1996b); (Kaasik *et al.*, 2001) Briefly, SR calcium loading was initiated at pCa 6.5 in the presence of exogenous ATP, with or without endogenous mitochondrial ATP production and/or CK-system activation. Azide (2 mM) was used to block mitochondrial ATP production, and solutions lacking phosphocreatine (PCr) were used to block creatine kinase function. SR calcium release was elicited with 5 mM caffeine, and was detected by measuring the resulting contractile force transient. At the end of the experiment pCa/tension relationship was assessed by stepwise increase in calcium concentration in the presence of caffeine. The calcium concentration that gave half maximal tension and the Hill coefficient were obtained by non-linear fit to the Hill equation $T=[Ca^{2+}]^{n_H}/(K+[Ca^{2+}]^{n_H})$, where T is the relative rigor tension, K a constant, and $n_H$ the Hill coefficient, and used to calculate the $[Ca^{2+}]$-time integral as an index of SR calcium load (De Sousa *et al.*, 1999). The ATP-channeling solution (ATP+MITO) contained (in mM): ethylene glycol-bis(ß-aminoethyl ether)N,N,N',N'-tetra-acetic acid 10 (EGTA), 0.2 during calcium release; N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid 40 (BES, pH 7.1); free $Mg^{2+}$ 1; taurine 10; glutamic acid 5; malic acid 2; $K_2HPO_4$ 3; dithiothreitol 0.5; $P^1,P^5$ diadenosine pentaphosphate 0.04 (to inhibit adenylate kinase activity); ruthenium red 0.005 (to block mitochondrial calcium uptake); MgATP 3.16; ionic strength was adjusted to 160 with potassium methanesulfonate, and 6% dextran was added to maintain normal cell volume after skinning.

Finally, we tested the ability of glycolysis to increase further the SR function (Boehm *et al.*, 2000), with further addition of 4 mM glyceraldehyde-3-phosphate (GAP), 4 mM of phosphoenolpyruvate (PEP) and 4 mM of nicotinamide adenosine dinucleotide (NAD).

***pMgATP-rigor tension relationships in saponin-permeabilized cardiac fibers***

pMgATP-rigor tension relationships were established by measuring the change in force during stepwise decreases in ATP concentration, until maximum rigor tension was obtained, with ATP alone, and with or without PCr and azide. Data were fitted using the Hill equation: $T=K/(K+[MgATP]^{n_H})$ where T is the relative rigor tension, K a constant, and $n_H$ the Hill coefficient. This fitting procedure was used to calculate $pMgATP_{50}$, at which half maximum rigor tension is obtained. Efficacy of ATP/ADP ratio control by mitochondrial ATP channeling and CK was calculated as the difference between $pMgATP_{50}$ with and without mitochondrial or CK activation.

***Confocal imaging of spontaneous $Ca^{2+}$ waves in saponin-skinned cardiac myocytes***

SR calcium uptake for the different energy loading conditions was also estimated by analyzing spontaneous $Ca^{2+}$ release from the SR with Fluo-4 fluorescence (excitation wavelength 488 nm; emission 500-595 nm) (Yang & Steele, 2002). Ventricular myocytes were isolated from sham and HF adult rats as described before (Verde *et al.*, 1999). For HF rats, 1 mg/ml hyaluronidase was added to facilitate tissue digestion. Cardiomyocytes were permeabilized with saponin (50 mg/ml) at room temperature (T = 22°C) for 3 min using basic internal solution (pCa 9), and then plated onto coverslips coated with Matrigel (Sigma) for attachment. Coverslips were attached on the stage of a Carl Zeiss LSM-510 confocal microscope using a recording chamber (Warner instruments, Hamden, CT, USA). Loading solutions were identical to those used for the permeabilized fiber experiments, except that [EGTA] was decreased to 100 μM (calcium concentration was estimated at 360 nM with calcium dye), and 4 μM Fluo-4 was added (Invitrogen). Images were acquired in line scan mode (at intervals of 3.5 or 7 ms) along the longitudinal axis of the cell. All the experiments were performed at 22°C.

***Biochemical analysis and immunoblotting***

Activities of total CK, citrate synthase (CS) and mitochondrial complex IV (COX) were measured using standard assays as previously described (De Sousa *et al.*, 1999). For immunoblotting, protein extracts (50 μg) were separated on 8% SDS-polyacrylamide gels and subsequently transferred to Hybond nitrocellulose membranes (Amersham). Membranes were incubated for 2 hours with antibodies against mitochondrial CK (mi-CK, gift from Dr Z. Khuchua, Nashville, TN, USA), SERCA2 (Santa Cruz Biotechnology Inc.California, USA) and calsequestrin (AffinityBioReagent, Golden, CO, USA). Chemiluminescence was detected using an ECL+ kit (Amersham) and quantified using an image-analysis system (Bio-Rad). Calsequestrin was used to normalize the protein expression.

***Statistics***

Data are expressed as mean±SEM. Statistical differences between two or more groups were determined by analysis of variance (ANOVA), or by one-way ANOVA with repeated measures (for different SR loading conditions), followed by Newman-Keuls post-hoc test. Significance was defined by a p-value less than 0.05 (95% confidence).

## Results

### *Anatomical Data*

After nearly five months of aortic banding, mortality was ≈ 60% in the HF group. The remaining HF animals showed decreased body weight, increased absolute heart weight and increased weights of heart, right and left ventricles and lungs normalized to tibia length, indicating severe heart failure (Table 1). Other anatomical signs of cardiac decompensation (ascites, pleural effusion, and edema) confirmed the presence of severe HF in this model.

### *Myofibrillar function of HF rats in saponin-permeabilized fibers*

Myofibrillar function was assessed in saponin skinned cardiac fibers from sham and HF rats (Table 2). Active tension at pCa 4.5 (defined as the difference between maximal and resting tension) was 22% lower in HF rat heart than sham ($p<0.001$), indicating contractile dysfunction. Passive tension was three-fold higher in saponin-skinned HF fibers than sham. Calcium sensitivity was unchanged.

### *Metabolic enzymes involved in energy production and consumption*

Enzyme activities of CK, CS and COX were decreased in banded rat heart by 38% ($p<0.01$), 24% ($p<0.05$) and 22% ($p<0.05$), respectively (Figure 1, panel A and B). Because decreased mitochondrial CK (mi-CK) activity is a good indicator of the severity of the pathology,(Nascimben *et al.*, 1996) we also estimated mi-CK expression by Western blot (Figure 1, panel B). Compared to sham, mi-CK expression was decreased by 59% ($p<0.005$). These results confirmed that mitochondrial energy production and CK-mediated energy transfer were reduced in our heart failure model, consistent with previous data showing impairment of maximal oxygen consumption rate.(De Sousa *et al.*, 1999)

Western Blot analysis showed that SERCA2 levels were unchanged in HF left ventricle (Figure 1, panel C).

### *Failing cardiomyocyte ultrastructure*

Compared with sham, the cytoarchitecture of failing rat heart myocytes was greatly disorganized, which made difficult quantification of ultrastructural images (Figure 2). Myofibrils and mitochondria were affected; there were regions where the longitudinal orientation of myofibrils was lost, and regions that had widened, zig-zagging Z lines with fewer contacts between mitochondria and myofibrils. In these regions, it was very difficult to

identify the membrane of the longitudinal sarcoplasmic reticulum. The size and distribution of mitochondria in failing myocytes were very heterogeneous. Mitochondrial clusters were present in the intermyofibrillar space, and in these clusters mitochondrial diameter varied greatly. Some mitochondria were small and round, whilst others were long and tubular, spanning over more than two sarcomeres. In some large mitochondria, a circular arrangement of cristae was evident, together with signs of mitochondrial degradation.

***Energy regulation of sarcoplasmic reticulum ATPase (SERCA) function***

*Calcium wave experiments on saponin-permeabilized cardiac myocytes*

In saponin-skinned cardiac myocytes spontaneous calcium waves can be detected at low EGTA concentration, due to cyclic uptake and release of $Ca^{2+}$ from the SR (Yang & Steele, 2002) (Figure 3, panel A). Wave frequency and decay time-course of the calcium waves were highly dependent on the ability of mitochondria and CK to regulate SERCA. In sham myocytes, ATP alone as energy-source was a poor substrate for SR calcium loading, because $T_{1/2}$ (the half-time of the decay phase) was almost 3-fold higher and wave frequency 3-fold lower in the absence of CK or mitochondrial substrates, indicating lower SR calcium content and less efficient calcium reuptake by SERCA. In HF myocytes, support by ATP diffusion alone remained poor (no significant change compared to sham), indicating no major change of intrinsic properties of SR in our conditions (Figure 3, panel B and C). However, calcium wave frequency was lower with CK and/or mitochondria substrates, and the descending phase of calcium waves was prolonged indicating lower calcium loading capacity of SERCA in HF vs. sham rats in the presence of CK and mitochondrial substrates.

*Mechanical experiments on saponin-permeabilized cardiac fibers*

SR calcium content was also assessed in saponin-permeabilized sham and HF rat cardiac fibers, using the force transient induced by caffeine stimulation (Kaasik *et al.*, 2001; Wilding *et al.*, 2006). SR calcium loading over five minutes was performed in the presence of ATP and with CK and/or mitochondrial substrates. Caffeine-induced tension transients had decreased amplitude in HF cardiac fibers under all energetic conditions (Figure 4A), consistent with reduced maximal active tension and myofibrillar disarray (Table 2) and with the cytoarchitecture disorganization. Calcium concentration-time transient (SCa), calculated using the force-calcium relationship for each fiber (see Methods), was used as an index of SR calcium content. Compared to sham, SCa was decreased in HF fibers by 43% ($p<0.005$) after loading with ATP+Mito+CK solution (Figure 4B), by 40% ($p<0.05$) with mitochondrial direct

ATP channeling (ATP+Mito), and by 49% ($p<0.005$) with bound creatine kinase (ATP+CK) (Figure 4B). Loading supported by exogenous ATP was again not significantly decreased.

In order to test whether altered phosphorylation of proteins such as SERCA, phospholamban or the ryanodine receptor played a role in impaired SR calcium loading in our HF model, we pre-incubated some fibers for one hour with 200 U/ml alkaline phosphatase which causes protein dephosphorylation. Dephosphorylation had no significant effect on calcium loading in sham fibers, and did not restore SR loading to sham levels in HF fibers (Figure 4C).

Finally, we tested whether SR function could be improved by the presence of an additional energy source (Figure 4B). In sham fibers, activation of supplementary glycolytic pathways increased SR loading from 36±3 to 57±3 μM.s (+55%). In HF fibers, the same level of SR loading was reached (57±8 μM.s), but the relative increase was larger (+155%), thus compensating for mitochondrial and CK impairment.

***Function of actomyosin-ATPase***

Rigor experiments were performed to assess the ability of mitochondrial direct ATP channeling and CK to control the ATP/ADP ratio in the vicinity of actomyosin-ATPase (myosin-ATPase). In the absence of calcium and PCr, and in the presence of azide (i.e. with non-working CK and mitochondria), a progressive decrease in MgATP concentration led to the development of rigor tension (Figure 5A). The pMgATP that produced half-maximal rigor tension ($pMgATP_{50}$) was shifted towards slightly higher values in HF, indicating greater accessibility of exogenous ATP to myosin-ATPase than in sham (Figure 5B). When mitochondria or CK were activated (by removing azide or by adding PCr, respectively), $pMgATP_{50}$ was increased due to local rephosphorylation of ADP by mitochondria or bound CK. This is the first demonstration that mitochondrial direct ATP channeling is effective at inhibiting rigor tension development in cardiac fibers. In HF fibers, the shift of $MgATP_{50}$ was smaller than in shams, indicating a reduced efficacy of local ATP regeneration by mitochondria and CK. Mitochondrial efficacy declined from 1.30±0.09 in sham to 0.75±0.08 in HF ($n=9$, $p<0.001$), whereas CK efficacy declined to a lesser extent, from 1.48±0.11 in shams to 1.08±0.09 in HF ($n=9$, $p=0.02$; Figure 5C). There was no statistical difference in the value of the Hill coefficient between groups, which ranged from 1.3 to 2.3.

***Relative contribution of CK and mitochondria for ATPases regulation***

We compared the relative efficacy of mitochondria and CK in regulating SERCA and myosin-ATPase in sham and HF rats (Figure 6). For this purpose, we calculated the ratio between mitochondria and CK efficacy relative to ATP, using the data from Fig. 4 and Fig. 5. Both mitochondria and CK appeared to contribute equally to local ATP regeneration near the myofibrils and SR in sham rats. Interestingly, in HF rats, the relative contribution of both energetic systems to SR regulation remained the same, but, a relative decrease in the mitochondrial contribution was observed ($p<0.05$) with respect to myofilaments regulation.

**Discussion**

The aim of this work was to determine whether the ability of CK and mitochondria to control the ATP/ADP ratio in the vicinity of two main cardiac cellular ATPases, SERCA and myosin-ATPase, is modified in HF and whether this might contribute to cardiomyocyte dysfunction. Using two different approaches in saponin-skinned fibers and cells, we observed a global decrease in the efficacy of both energetic systems: in the presence of functional CK and/or mitochondria, SR calcium loading was lower in HF vs. sham, and could be restored to normal level only by activation of bound glycolytic enzymes. For myofibrils, CK and mitochondria were also less efficient in HF than in sham myocardium, causing contractile dysfunction. Energy deficiency and impairment of local control of ATP/ADP may thus contribute to the global contractile dysfunction in heart failure.

***Methodological approaches to the study of local energy regulation***

To investigate local energy regulation near SERCA and myofilament ATPases in a physiological setting, we used a unique method developed ten years ago in our laboratory (Minajeva *et al.*, 1996). It is based on the use of sarcolemmal membrane permeabilization with saponin which preserves the integrity of the cellular architecture and organelles, and allows a precise control of the composition of the intracellular medium. The intracellular medium is kept physiological and can be modified to control bulk energy supply, calcium concentration, pH, reactive oxygen species, and ion concentrations. Moreover, the ability of each energy source to control local ATP/ADP ratio can be investigated separately or in combination.

To assess SERCA function, we combined two complementary experimental approaches: in the first one, we used myofilaments from skinned fibers as an intracellular functional probe for calcium released by the SR; in the second one, we used skinned cardiomyocytes loaded with a fluorescent calcium indicator. In the fibers, SR calcium uptake

capacity was assessed by recording the time integral of the tension transient induced by caffeine in the presence of optimal and constant energy supply (ATP+Mito+CK). A major advantage of this is that the myofilaments respond to "the functional calcium", i.e. the calcium stored into the SR that can be mobilized and can reach myofilaments upon full activation of the ryanodine receptors. To circumvent possible confounding effects due to intrinsic myofibrillar changes in heart failure, the calcium sensitivity of the myofilaments was assessed for each fiber providing a tension-pCa calibration curve from which the amount of released calcium was calculated (Minajeva *et al.*, 1996). In the skinned isolated myocytes, spontaneous SR calcium release and reuptake were elicited in the presence of a sub-threshold calcium concentration. The resulting intracellular calcium waves develop with a frequency and $T_{1/2}$ which reflect the efficacy of calcium uptake into the SR (Yang & Steele, 2002). Because the composition of the bulk cytosol can be precisely controlled, this approach allowed evaluating the consequences on SR calcium release of a change in local concentrations of nucleotides or energy sources.

To assess energy compartmentation in myofilaments, we used a well-established approach of the dependency of rigor tension development on local control of ATP/ADP by CK in triton-X100 (Ventura-Clapier *et al.*, 1987). This approach was modified using saponin instead of triton-X100, which allowed us for the first time to evaluate the role of mitochondrial energy supply.

***Local energetic regulation of ATPases is impaired in the failing rat heart***

Even when energy stores are kept constant, a decreased capacity for local energy regeneration may lead to dysfunction of cellular ATPases. In sham fibers or cardiomyocytes, ATP alone is not sufficient to sustain SR calcium load, and local withdrawal of ADP and regeneration of ATP by either CK or mitochondria or both (Kaasik *et al.*, 2001) is necessary for efficient thermodynamic and kinetic control of ATPases. This is evidence that SR calcium loading capacity, and thus SR calcium content, is also dependent on local energy supply. In heart failure, SR loading was not different when ATP alone was provided. However, when the CK and/or mitochondrial systems were activated, SR loading was lower, indicating a further limitation of the energy supply due to local regeneration systems. The same holds true for cardiac myofilaments in HF, where either mitochondria- or CK-supported myofibrillar function was impaired. This impairment occurs despite a sharp decrease in the proportion of the fast myosin isoform in the failing heart, which should increase the economy of contraction (Herron & McDonald, 2002) and reduce energy demand. Altogether, these results indicate

that local energy limitation occurs in HF which affects both calcium homeostasis and myofibrillar function.

An impaired local ATP/ADP ratio may not be the only reason for the observed decreased SR calcium release in HF. Although not always observed (Frank *et al.*, 2002), there is evidence for a SERCA2 down-regulation in HF. In our model of heart failure, cardiac SERCA2 expression was normal. In the presence of ATP alone, no difference was observed in calcium loading between sham and HF. Interestingly, maximal activation of glycolysis was sufficient to restore a normal SR loading (i.e. HF matched sham levels), indicating 1) that energy supply is a limiting factor for SR function in HF, and 2) that glycolysis plays a more important role in HF in supporting SR function, to compensate for the impairment of CK (like it occurs in skeletal muscle, (Janssen *et al.*, 2003) and mitochondria. Glycolysis may also act by decreasing SR calcium leak (Zima *et al.*, 2006) thus preserving the balance between load and leak, in the presence of all energy sources.

In the recent years it has been emphasized that calcium leak due to hyperphosphorylation of ryanodine receptors or reduced phosphorylation level of phospholamban may participate in the decreased SR calcium content and calcium transient in heart failure (Frank *et al.*, 2002); (Lehnart *et al.*, 2005). However, alkaline phosphatase had no significant effect on calcium loading in our experiments, suggesting that our results were not due to a change in SERCA expression level and/or protein phosphorylation. Yet, the contribution of phosphorylation may be underscored in our experiments because the permeabilization procedure may in itself lead to protein dephosphorylation, which could explain why alkaline phosphatase had no significant effect. This does not rule out the role of phosphorylation on altered calcium fluxes but provide evidence that local energy regulation could additionally take part in the defective SR function in HF. This is in line with the similar results obtained with myofibrillar ATPase where HF induced alterations in local control of energy supply by mitochondria and CK directly impacts myofibrillar function.

***Cytoarchitecture and heart failure***

In cardiac myocytes, specialized cellular functions are highly organized within structural and functional compartments (Saks *et al.*, 1994).(Ventura-Clapier *et al.*, 1998; Weiss & Korge, 2001) Each subcellular compartment is a functional and structural entity (Saks *et al.*, 2001). The role of the cytoskeleton in stabilizing cellular structure and functional integrity is well established.(Clark *et al.*, 2002) In heart failure, profound alterations of microtubules or intermediate filament protein expression may contribute to functional

impairment (Hein *et al.*, 2000).(Belmadani *et al.*, 2002; Wilding *et al.*, 2005; Cooper, 2006) It was proposed that an increased distance between the calcium channel and RyR may affect the efficiency of excitation-contraction coupling in HF (Gomez *et al.*, 1997). Cytoskeletal organization may also play a role in the regulation of energy exchanges (Milner *et al.*, 2000); (Appaix *et al.*, 2003), since it is required for positioning mitochondria and anchoring myofibrils (Capetanaki, 2002). A recent study by our laboratory focused on two models of cytoarchitectural disorganization, the muscle LIM protein (MLP) and the desmin knock-out mouse: in both models, a specific alteration of mitochondria-SR interaction was observed (Wilding *et al.*, 2006), suggesting that efficient energy transfer depends on the arrangement of organelles and myofibrils, independently of mitochondrial function and SR content.

In the present study, as in other models of heart failure (Sabbah *et al.*, 1992), we found misalignment of mitochondria and myofibrils, heterogeneity of mitochondrial shape and size and mitochondrial degradation. These changes could be the basis for the decreased energy transfer between mitochondria and myosin-ATPase in addition to the decrease in energy production. Indeed, while in sham rats both CK and mitochondria contribute equally to SR and myofibrillar functions (Figure 6), in HF heart, mitochondria were less efficient than CK in maintaining an adequate energy supply for contraction. Our data suggest that energetic remodeling occurs in HF, which alters the ratio of efficacy between the two main energy sources. Due to the large structure of myofibrils, the core of myofilaments may be more dependent on mitochondrial form, mass, and architectural arrangement than SR which is in closer physical interaction with mitochondria. On the other hand, because CK is closely bound to myofilaments and SR, CK efficacy will depend more on the amount of bound CK than on architectural design. Thus, in HF, mitochondria could be more limiting than CK for the regulation of ATP/ADP ratio in the vicinity of myofibrils as compared to SR. Maintaining the close interaction between mitochondria and myofibrils appears to be crucial for optimal energetic regulation.

**Implications for cardiac function**

In vivo, the reduction in myocardial CK fluxes, the decreased in fatty acid utilization and the decreased oxidative capacity are sufficient to compromise ATP buffering capacity and limit ATP availability to the myofibrils and SR calcium pump, and in turn affect both systolic and diastolic function (Ten Hove & Neubauer, 2007; De Sousa *et al.*, 1999; Smith *et al.*, 2006). Consequently, and in contrast to our experimental conditions, *in vivo* the concentrations of substrates and intermediates are no longer in excess, further enhancing

energetic limitations. The importance of glycolytically produced substrates for mitochondrial oxidative phosphorylation is increased, but remains limited particularly under conditions of high workload (Ventura-Clapier *et al.*, 2004). Impairment of CK- or mitochondria-mediated control of ATPases via the local ATP/ADP ratio demonstrated here will participate in impairment of SR calcium handling and contraction and thus excitation-contraction coupling. Importantly, in the healthy heart the two main energy-supplying systems (CK and direct mitochondrial energy channeling) show clear redundancy, creating a certain reserve in situations of increased energy demands of high workload. However, this is not anymore the case in the failing heart as demonstrated here. Thus, the failing heart seems to be unable to meet high hemodynamic requirements of the body because in part of lack of chemical energy available, defective energy distribution, and energy starvation (Ingwall & Weiss, 2004; Neubauer, 2007)

In conclusion, the present data suggest that there are intimate links between cytoarchitecture, energy metabolism and contractile activity ensuring efficient cardiac pump function. Depression of contractile force and myocardial function in heart failure may result from intricate alterations of calcium handling, cytoarchitectural disorganization and energetic starvation. Further experiments are needed in order to understand better the link between energy, cytoarchitecture and contraction and the need to maintain these components in harmony to ensure optimal cardiac cell function.

## References


Appaix F, Kuznetsov AV, Usson Y, Kay L, Andrienko T, Olivares J, Kaambre T, Sikk P, Margreiter R & Saks V (2003). Possible role of cytoskeleton in intracellular arrangement and regulation of mitochondria. *Exp Physiol* **88**, 175-90.

Belevych A, Kubalova Z, Terentyev D, Hamlin RL, Carnes CA & Gyorke S (2007). Enhanced ryanodine receptor-mediated calcium leak determines reduced sarcoplasmic reticulum calcium content in chronic canine heart failure. *Biophys J* **93**, 4083-92.

Belmadani S, Pous C, Ventura-Clapier R, Fischmeister R & Mery PF (2002). Post-translational modifications of cardiac tubulin during chronic heart failure in the rat. *Mol Cell Biochem* **237**, 39-46.

Bers DM (2006). Altered cardiac myocyte Ca regulation in heart failure. *Physiology (Bethesda)* **21**, 380-7.

Boehm E, Ventura-Clapier R, Mateo P, Lechene P & Veksler V (2000). Glycolysis supports calcium uptake by the sarcoplasmic reticulum in skinned ventricular fibres of mice deficient in mitochondrial and cytosolic creatine kinase. *J Mol Cell Cardiol* **32**, 891-902.

Capetanaki Y (2002). Desmin cytoskeleton: a potential regulator of muscle mitochondrial behavior and function. *Trends-Cardiovasc-Med* **12**, 339-48.

Clark KA, McElhinny AS, Beckerle MC & Gregorio CC (2002). Striated muscle cytoarchitecture: an intricate web of form and function. *Annu-Rev-Cell-Dev-Biol* **18**, 637-706.

Cooper G 4th (2006). Cytoskeletal networks and the regulation of cardiac contractility: microtubules, hypertrophy, and cardiac dysfunction. *Am J Physiol Heart Circ Physiol* **291**, H1003-14.

De Sousa E, Veksler V, Minajeva A, Kaasik A, Mateo P, Mayoux E, Hoerter J, Bigard X , Serrurier B & Ventura-Clapier R (1999). Subcellular creatine kinase alterations. Implications in heart failure. *Circ Res* **85**, 68-76.

Dzeja PP, Redfield MM, Burnett JC & Terzic A (2000). Failing energetics in failing hearts. *Curr-Cardiol-Rep* **2**, 212-7.

Dzeja PP & Terzic A (2003). Phosphotransfer networks and cellular energetics. *J Exp Biol* **206**, 2039-47.

Frank KF, Bolck B, Brixius K, Kranias EG & Schwinger RH (2002). Modulation of SERCA: implications for the failing human heart. *Basic Res Cardiol* **97 Suppl 1**, I72-8.

Garnier A, Fortin D, Delomenie C, Momken I, Veksler V & Ventura-Clapier R (2003). Depressed mitochondrial transcription factors and oxidative capacity in rat failing cardiac and skeletal muscles. *J Physiol* **551**, 491-501.

Gomez AM, Valdivia HH, Cheng H, Lederer MR, Santana LF, Cannell MB, McCune SA, Altschuld RA & Lederer WJ ( 1997). Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure . *Science* **276**, 800-6.

Hein S, Kostin S, Heling A, Maeno Y & Schaper J (2000). The role of the cytoskeleton in heart failure. *Cardiovasc-Res* **45**, 273-8.

Herron TJ & McDonald KS (2002). Small amounts of alpha-myosin heavy chain isoform expression significantly increase power output of rat cardiac myocyte fragments. *Circ Res* **90**, 1150-2.

Ingwall JS & Weiss RG (2004). Is the failing heart energy starved? On using chemical energy to support cardiac function. *Circ-Res* **95**, 135-45.

Janssen E, Terzic A, Wieringa B & Dzeja PP (2003). Impaired intracellular energetic communication in muscles from creatine kinase and adenylate kinase (M-CK/AK1) double knock-out mice. *J Biol Chem* **278**, 30441-9.

Kaasik A, Veksler V, Boehm E, Novotova M, Minajeva A & Ventura-Clapier R (2001). Energetic crosstalk between organelles: architectural integration of energy production and utilization. *Circ Res* **89**, 153-9.

Lehnart SE, Wehrens XH & Marks AR (2005). Defective ryanodine receptor interdomain interactions may contribute to intracellular Ca2+ leak: a novel therapeutic target in

heart failure. *Circulation* **111**, 3342-6.

Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblit N & Marks AR (2000). PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* **101**, 365-76.

Milner DJ, Mavroidis M, Weisleder N & Capetanaki Y (2000). Desmin cytoskeleton linked to muscle mitochondrial distribution and respiratory function. *J-Cell-Biol* **150**, 1283-98.

Minajeva A, Ventura-Clapier R & Veksler V (1996). Ca2+ uptake by cardiac sarcoplasmic reticulum ATPase in situ strongly depends on bound creatine kinase. *Pflugers Arch* **432**, 904-12.

Nascimben L, Ingwall JS, Pauletto P, Friedrich J, Gwathmey JK, Saks V, Pessina AC & Allen PD (1996). Creatine kinase system in failing and nonfailing human myocardium. *Circulation* **94**, 1894-901.

Neubauer S (2007). The failing heart--an engine out of fuel. *N Engl J Med* **356**, 1140-51.

Neubauer S, Horn M, Cramer M, Harre K, Newell JB, Peters W, Pabst T, Ertl G, Hahn D, Ingwall JS & Kochsiek K (1997). Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. *Circulation* **96**, 2190-6.

Sabbah HN, Sharov V, Riddle JM, Kono T, Lesch M & Goldstein S (1992). Mitochondrial abnormalities in myocardium of dogs with chronic heart failure. *J Mol Cell Cardiol* **24**, 1333-47.

Saks V, Dzeja P, Schlattner U, Vendelin M, Terzic A & Wallimann T (2006). Cardiac system bioenergetics: metabolic basis of the Frank-Starling law. *J Physiol* **571**, 253-73.

Saks VA, Kaambre T, Sikk P, Eimre M, Orlova E, Paju K, Piirsoo A, Appaix F, Kay L, Regitz-Zagrosek V, Fleck E & Seppet E (2001). Intracellular energetic units in red muscle cells. *Biochem J* **356**, 643-57.

Saks VA, Khuchua ZA, Vasilyeva EV, Belikova OYu & Kuznetsov AV (1994). Metabolic compartmentation and substrate channelling in muscle cells. Role of coupled creatine kinases in in vivo regulation of cellular respiration--a synthesis. *Mol Cell Biochem* **133-134**, 155-92.

Smith CS, Bottomley PA, Schulman SP, Gerstenblith G & Weiss RG (2006). Altered creatine kinase adenosine triphosphate kinetics in failing hypertrophied human myocardium. *Circulation* **114**, 1151-8.

Ten Hove M & Neubauer S (2007). MR spectroscopy in heart failure--clinical and experimental findings. *Heart Fail Rev* **12**, 48-57.

Ventura-Clapier R, Garnier A & Veksler V (2004). Energy metabolism in heart failure. *J-Physiol* **555**, 1-13.

Ventura-Clapier R, Kuznetsov A, Veksler V, Boehm E & Anflous K (1998). Functional coupling of creatine kinases in muscles: species and tissue specificity. *Mol Cell Biochem* **184**, 231-47.

Ventura-Clapier R, Mekhfi H & Vassort G (1987). Role of creatine kinase in force development in chemically skinned rat cardiac muscle. *J Gen Physiol* **89**, 815-37.

Verde I, Vandecasteele G, Lezoualc'h F & Fischmeister R (1999). Characterization of the cyclic nucleotide phosphodiesterase subtypes involved in the regulation of the L-type Ca2+ current in rat ventricular myocytes. *Br J Pharmacol* **127**, 65-74.

Weiss JN & Korge P (2001). The cytoplasm: no longer a well-mixed bag. *Circ Res* **89**, 108-10.

Weiss JN, Yang L & Qu Z (2006). Systems biology approaches to metabolic and cardiovascular disorders: network perspectives of cardiovascular metabolism. *J Lipid Res* **47**, 2355-66.

Wilding JR, Joubert F, de Araujo C, Fortin D, Novotova M, Veksler V & Ventura-Clapier R (2006). Altered energy transfer from mitochondria to sarcoplasmic reticulum after cytoarchitectural perturbations in mice hearts. *J Physiol* **575**, 191-200.

Wilding JR, Schneider JE, Sang AE, Davies KE, Neubauer S & Clarke K (2005). Dystrophin- and MLP-deficient mouse hearts: marked differences in morphology and function, but similar accumulation of cytoskeletal proteins. *FASEB J* **19**, 79-81.

Yang Z & Steele DS (2002). Effects of phosphocreatine on SR Ca(2+) regulation in isolated saponin-permeabilized rat cardiac myocytes. *J-Physiol* **539**, 767-77.

Zima AV, Kockskamper J & Blatter LA (2006). Cytosolic energy reserves determine the effect of glycolytic sugar phosphates on sarcoplasmic reticulum Ca2+ release in cat ventricular myocytes. *J Physiol* **517** Part 1, 281-93.

**Acknowledgments**

We thank Dr. R. Fischmeister for continuous support and critical reading of the manuscript, Florence Lefebvre for preparation of isolated cells, the animal core facility of IFR141 for efficient handling and preparation of the animals, and Valérie Nicolas for help with the confocal microscopy. This work was supported by the Institut National de la Santé et de la Recherche Médicale, La Fondation de France, The Leverhulme Trust (JRW), the Centre National de la Recherche Scientifique (FJ, RV-C), APVT –51-31104 and VEGA 2/3189/25 (MN), Ministère des Affaires Etrangères STEFANIK (08093WL), the European Union Contract n°LSHM-CT-2005-018833/EUGeneHeart.

**Table 1. Anatomical parameters in HF rats compared with sham.**

| | **sham (n=9)** | **HF (n=9)** | **P *** |
|---|---|---|---|
| Aortic banding duration (months) | 4.9±0.1 | 4.8±0.1 | NS |
| Body weight (g) | 533±23 | 429±16 | <0.001 |
| Heart weight (g) | 1.5±0.1 | 2.8±0.1 | <0.001 |
| Tibia length (cm) | 4.55±0.13 | 4.37±0.06 | NS |
| Heart weight / tibia length (g/cm) | 0.33±0.03 | 0.65±0.02 | <0.001 |
| Right ventricle weight / tibia length (g/cm) | 0.058±0.005 | 0.117±0.005 | <0.001 |
| Left ventricle weight / tibia length (g/cm) | 0.22±0.02 | 0.36±0.01 | <0.001 |
| Lung weight / tibia length (g/cm) | 0.40±0.02 | 1.02±0.08 | <0.001 |
| Liver weight / tibia length (g/cm) | 3.7±0.2 | 3.2±0.2 | 0.05 |

Values are mean±SEM; n=number of animals, * Statistical differences between HF and sham values; NS: non significant

**Table 2. Myofibrillar functions in saponin permeabilized cardiac fibers.**

| | **sham** | **HF** | **P *** |
|---|---|---|---|
| Active tension ($mN/mm^2$) | 37±2 | 29±2 | <0.001 |
| | (n=22) | (n=23) | |
| Resting tension ($mN/mm^2$) | 4.9±0.8 | 16.8±2.1 | <0.001 |
| | (n=22) | (n=23) | |
| $pCa_{50}$ | 5.82±0.01 | 5.83±0.01 | NS |
| | (n=12) | (n=13) | |
| $n_H$ | 4.8±0.2 | 4.3±0.1 | NS |
| | (n=12) | (n=13) | |

Values are mean±SEM; $pCa_{50}$: pCa for half maximal tension development. $n_H$: Hill coefficient; NS: non significant. * Statistical differences between HF and sham values. n = number of fibers

**Figures**

**Figure 1. Enzymes involved in energy production and consumption in sham and HF rats.**

A: Mitochondrial enzymes (CS, citrate synthase; COX, cytochrome oxidase; IU, µmol/min). B: CK content (total CK activity IU, µmol/min; mi-CK, western blot analysis of mitochondrial–CK; au: arbitrary units). Panel C: Representative western blot of SERCA2 and mean values of SERCA2 expression levels. Calsequestrin was used as loading control (n=8 in each group).

* $p<0.05$, ** $p<0.005$ vs. sham.

**Figure 2. Electron micrographs of papillary muscles from sham (A,C) and HF (B,D) rats.**

A – longitudinal section of cardiomyocyte from sham rat with regular organization of myofibrils and rows of mitochondria (arrows).

B – longitudinal section of cardiomyocyte from HF rat with irregular organization of myofibrils with small areas of the disorganized myofibrils (atsterisk) Mitochondria are extended along the long fiber axis (arrows) and form clusters in the intermyofibrillar space.

C – detail showing mitochondria regular of the shape (arrows) found close to the myofibrils in cardiomyocytes from sham rat.

D – portion of cardiomyocyte from HF rat. Intermyofibrillar space is rich in cytosol (arrow). In the myofibrils irregular course of the Z-line is seen (arrowhead).

**Figure 3. Confocal imaging of spontaneous $Ca^{2+}$ waves.**

**A:** Fluo-4 original (lower lane) and averaged (upper lane) signals from representative experiments showing spontaneous $Ca^{2+}$ waves in saponin-skinned sham cardiomyocytes in different energy solutions: ATP alone (ATP) or with mitochondrial and CK system activation (ATP+CK+MITO), mitochondria (ATP+MITO) or CK (ATP+CK). [EGTA] = 100 µM, pCa 6.5. Graphs showing the mean $Ca^{2+}$ wave frequency (B) and the half time of the descending phase of the wave ($T_{1/2}$, C) in sham and HF rat myocytes (n=10-15 cells for each condition from 3 different hearts).

**Figure 4. SR calcium loading under different energetic conditions in sham and HF permeabilized cardiac fibers.**

Representative tension transients during SR calcium release in sham (**Panel A**) and HF (**Panel B**) cardiac fibers. The SR was loaded with calcium for 5 min under different energetic conditions (ATP+Mito+CK, ATP+Mito, ATP+CK, ATP, see Methods).
C and D. Calcium load was estimated by relating tension transients, elicited by caffeine stimulation to the calcium-tension curve for each fiber (SCa). **Panel C**: energy supplied by ATP, by ATP+Mito, ATP+CK, ATP+mitos+CK, or by ATP+mitos+CK+ glycolysis (Gly). * $p<0.05$ vs Sham. **Panel D**: same experiments as in Panel C in the presence of alkaline phosphatase.

**Figure 5. Function of actomyosin-ATPase in sham and HF rat**

**Left part.** Graphs showing relative pMgATP/rigor tension relationship without or with substrates for mitochondria (**A**) and creatine kinase (**B**). pMgATP-rigor tension relationship is established by a stepwise decrease in ATP concentration (in the absence of calcium) until maximum rigor tension was obtained. The curves represent the Hill equation fitting of data (see Methods) obtained with the mean of seven experiments in ventricular skinned fibers from sham (continuous line) and HF rat fibers (dashed lines). The shift observed by addition of mitochondrial substrates (+MITO) or PCr (+CK) represent the efficacy of both systems for the regulation of actomyosin ATPase function. **C:** Means of $pMgATP_{50}$ (ATP concentration needed to obtain the half maximum rigor tension) estimated by rigor experiments. **D**: Mean values of efficacy of mitochondria and CK for myosin ATPase regulation. * $p<0.05$, ** $p<0.005$ vs. sham.

**Figure 6. Relative efficacy of mitochondria and CK for regulating SR and myofibrils function in sham and HF.**

For SR, the relative efficacy of energy sources was calculated as the ratio between SCa after loading in presence of mitochondrial substrates (ATP + MITO) and SCa in the presence of PCr (ATP + PCr) (data from figure 4). For myofilaments, the relative efficacy is calculated as the ratio between efficacy of mitochondria and CK for myosin ATPase regulation (from figure 5). In sham hearts, the efficacy of mitochondria for regulating local ATP/ADP is similar in SR and in myofilaments (compared to CK), but appears to be more affected in myofilaments than in SR in HF.

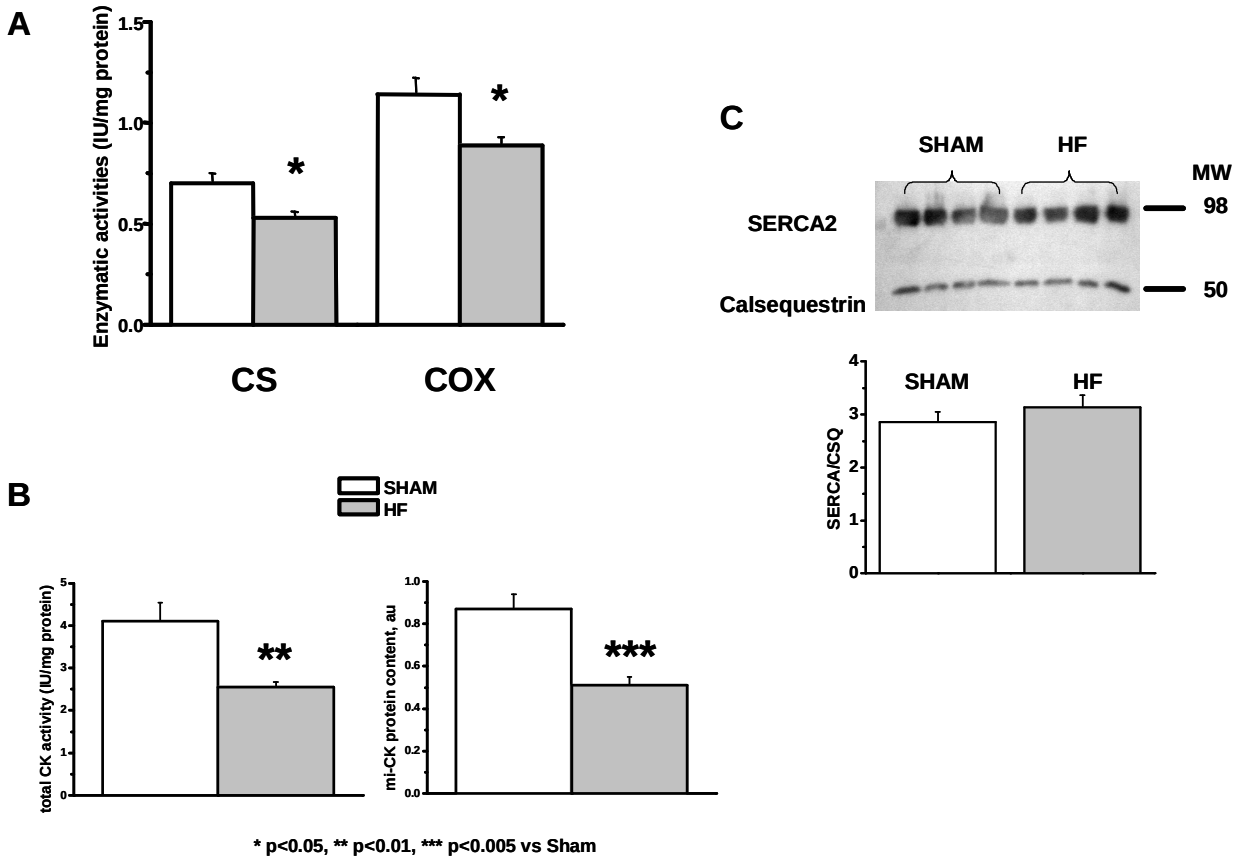

A
Enzymatic activities (IU/mg protein)
1.5
1.0
0.5
0.0
*
*
CS
COX
B
SHAM
HF
total CK activity (IU/mg protein)
5
4
3
2
1
0
**
mi-CK protein content, au
1.0
0.8
0.6
0.4
0.2
0.0
***
* p<0.05, ** p<0.01, *** p<0.005 vs Sham
C
SHAM
HF
MW
SERCA2
98
Calsequestrin
50
SERCA/CSQ
4
3
2
1
0
SHAM
HF

Figure 1

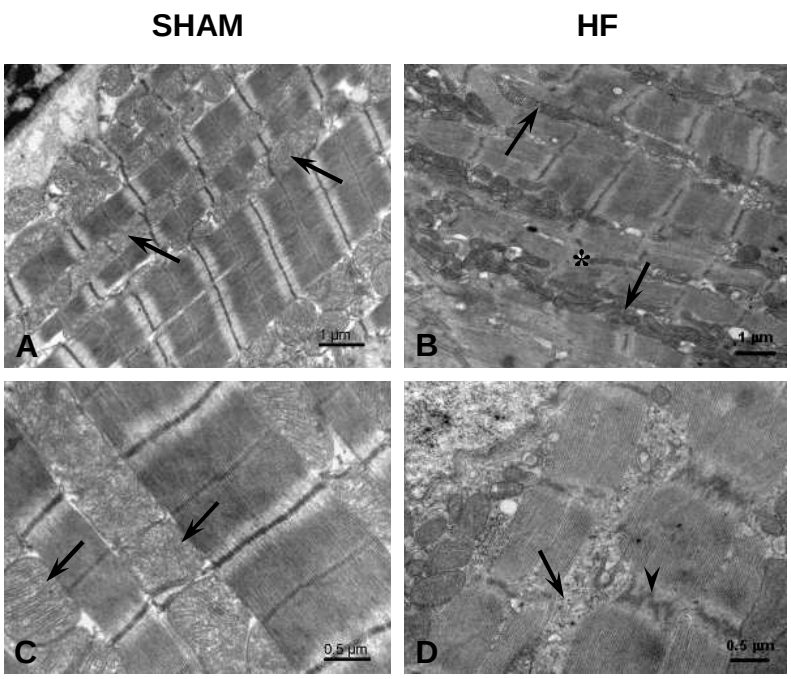



Figure 2

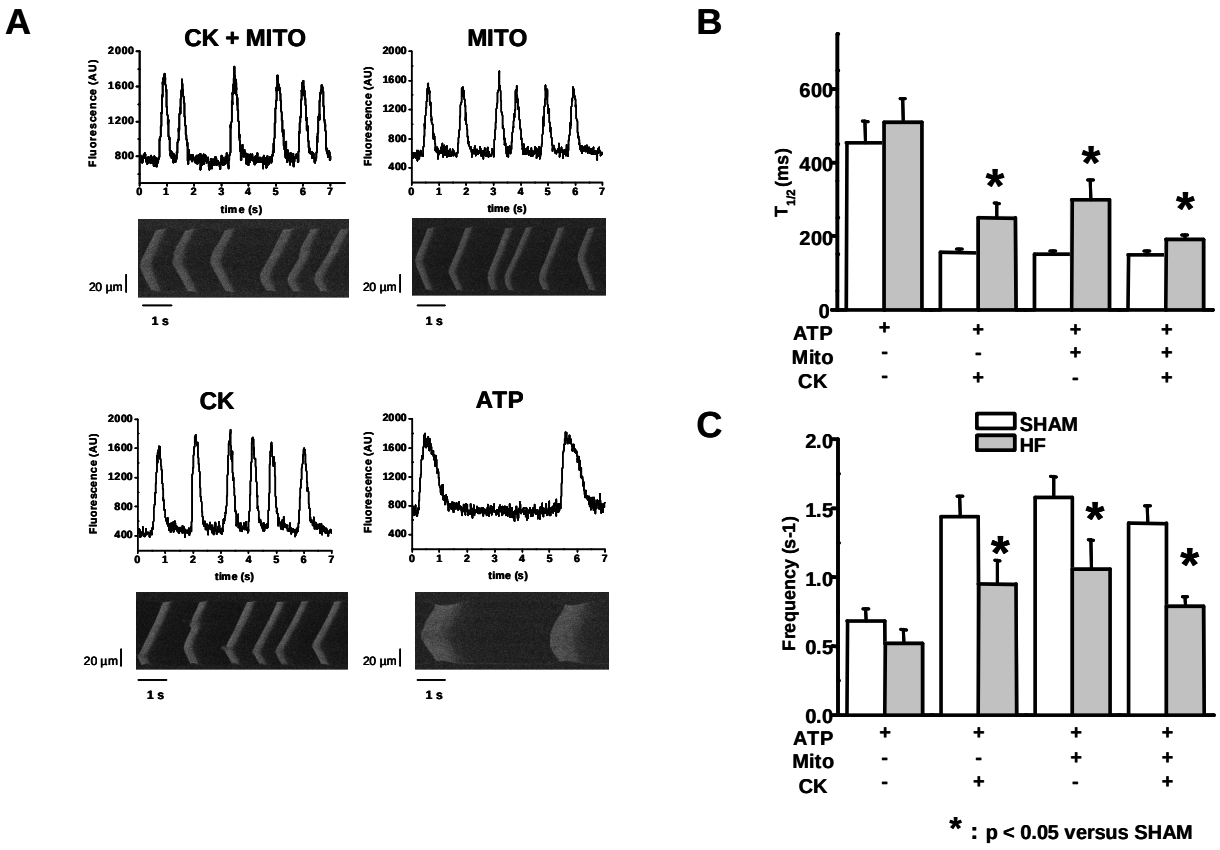

A
CK + MITO
MITO
CK
ATP
Fluorescence (AU)
time (s)
20 µm
1 s
B
$T_{1/2}$ (ms)
ATP
Mito
CK
C
SHAM
HF
Frequency (s-1)
* : p < 0.05 versus SHAM

Figure 3

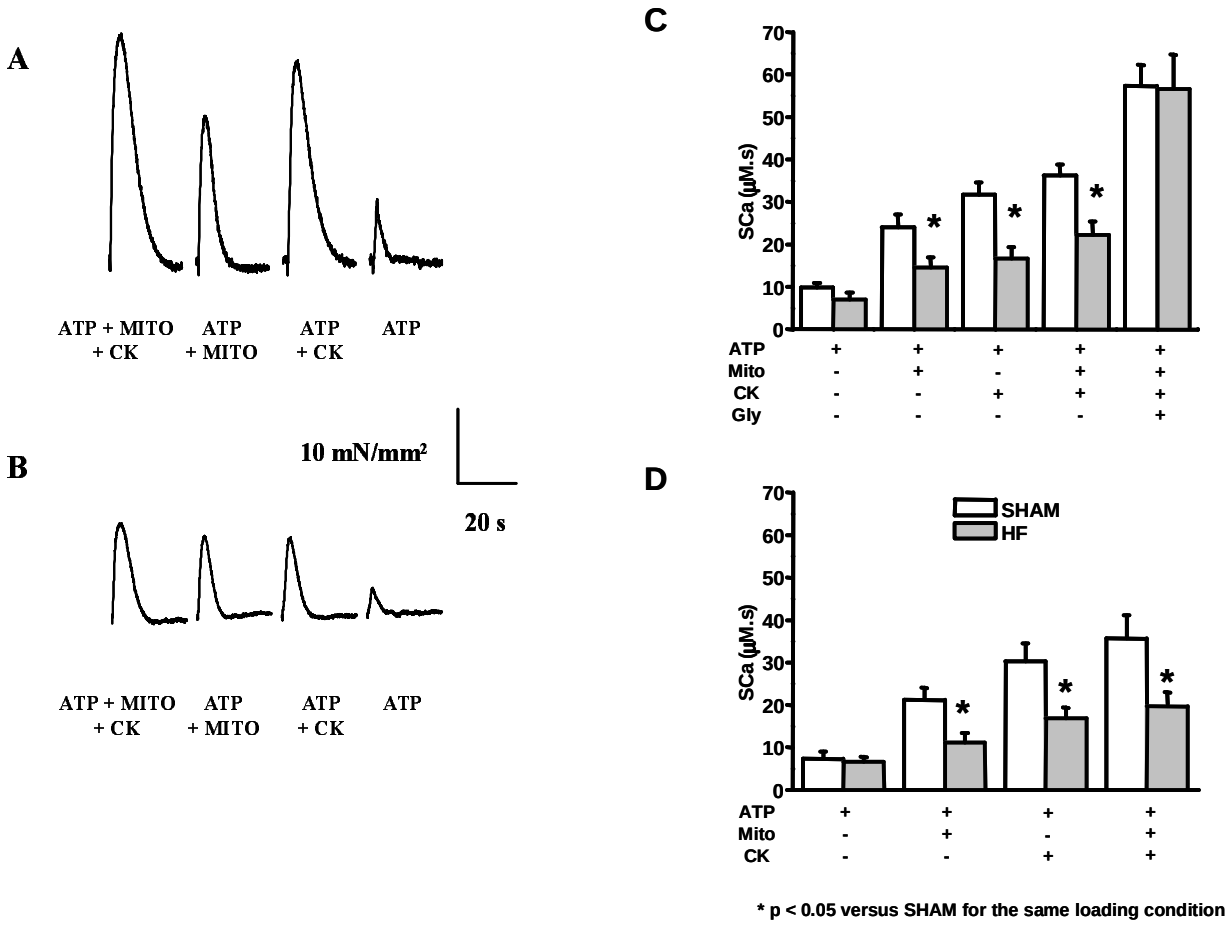

A
ATP + MITO + CK
ATP + MITO
ATP + CK
ATP
B
10 mN/mm²
20 s
ATP + MITO + CK
ATP + MITO
ATP + CK
ATP
C
SCa (µM.s)
70
60
50
40
30
20
10
0
ATP + + + + +
Mito - + - + +
CK - - + + +
Gly - - - - +
D
SHAM
HF
SCa (µM.s)
70
60
50
40
30
20
10
0
ATP + + + +
Mito - + - +
CK - - + +
* p < 0.05 versus SHAM for the same loading condition

Figure 4

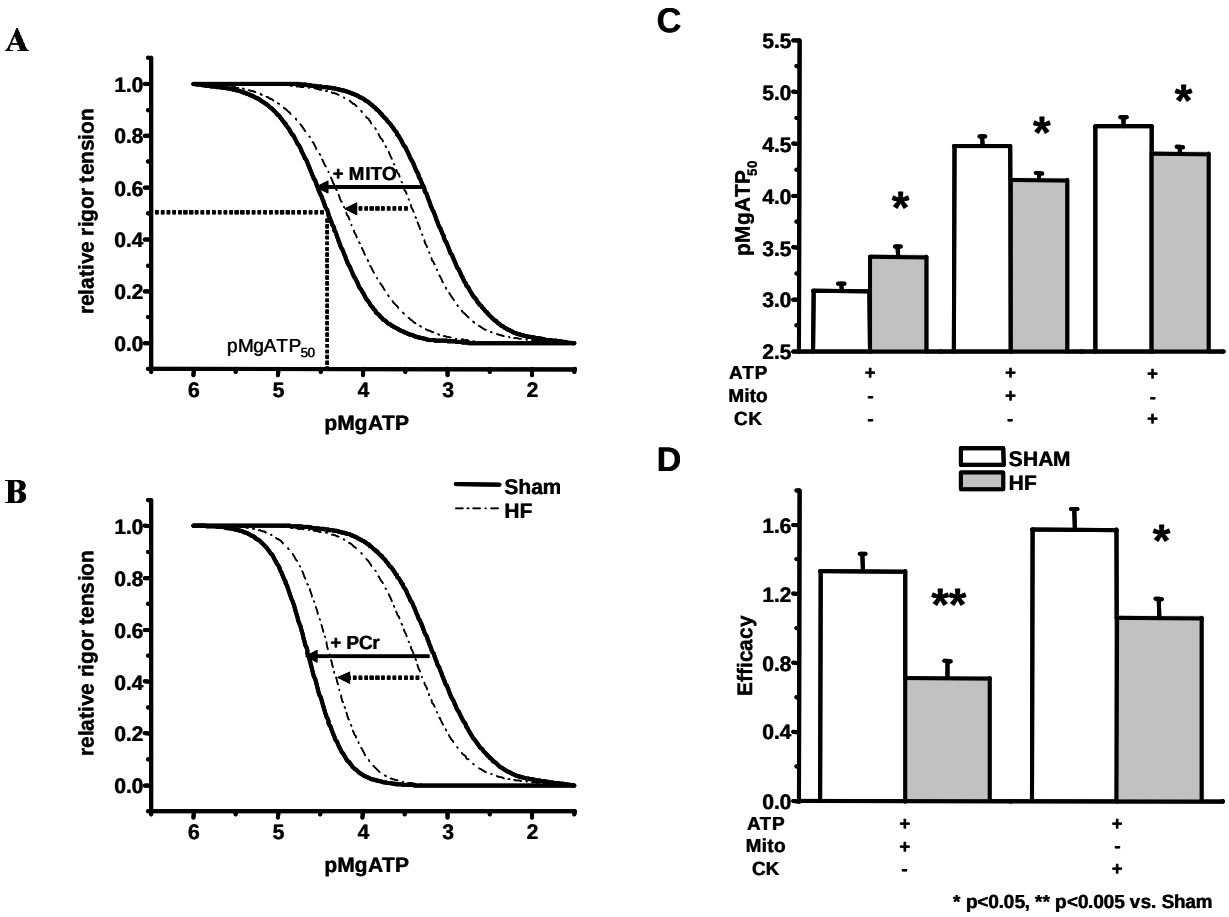

A
relative rigor tension
1.0
0.8
0.6
0.4
0.2
0.0
+ MITO
pMgATP50
6
5
4
3
2
pMgATP
B
Sham
HF
relative rigor tension
+ PCr
pMgATP
C
pMgATP50
5.5
5.0
4.5
4.0
3.5
3.0
2.5
*
ATP + + +
Mito - + -
CK - - +
D
SHAM
HF
Efficacy
1.6
1.2
0.8
0.4
0.0
**
*
ATP + +
Mito + -
CK - +
* p<0.05, ** p<0.005 vs. Sham

Figure 5

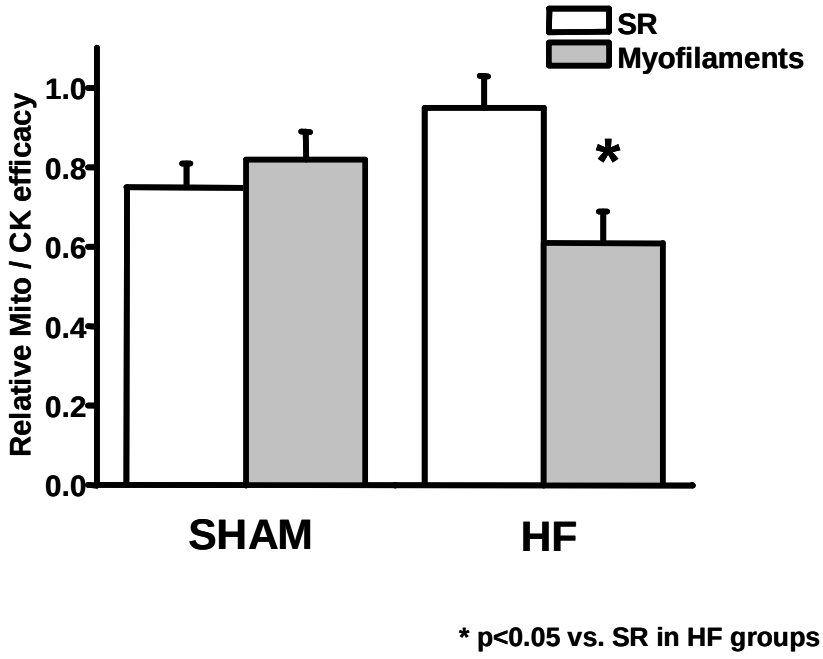

SR
Myofilaments
Relative Mito / CK efficacy
1.0
0.8
0.6
0.4
0.2
0.0
*
SHAM
HF
* p<0.05 vs. SR in HF groups

Figure 6