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Creatine kinase knockout mice show left ventricular hypertrophy and dilatation, but unaltered remodeling post-myocardial infarction

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Abstract

Objective: Creatine kinase (CK) is responsible for the transport of high-energy phosphates in excitable tissue and is of central importance in myocardial energy homeostasis. Significant changes in myocardial energetics have been reported in mice lacking the various CK isoenzymes. Our hypothesis was that ablation of CK isoenzymes leads to cardiac hypertrophy, impaired function, and aggravation of left ventricular remodeling post-myocardial infarction.

Methods: CK-deficient mice (CK KO) were examined by cardiac magnetic resonance imaging (MRI) to determine left ventricular volumes, ejection fraction, and mass: ten wild-type (WT), 6 mitochondrial CK KO (Mito-CK$^{-/-}$), 10 cytosolic CK KO (M-CK$^{-/-}$), and 10 mice with combined KO (M/Mito-CK$^{-/-}$).

Results: While ejection fraction was similar in all groups, there was significant LV dilatation with a ~30% increase in LV end-diastolic volumes in Mito-CK$^{-/-}$ and in M/Mito-CK$^{-/-}$. Compared to WT, there was a striking 73% and 64% increase of LV mass in Mito-CK$^{-/-}$ and in M/Mito-CK$^{-/-}$ mice, respectively, but no significant increase of LV mass (+33%; p=n.s.) in M-CK$^{-/-}$. Furthermore, significant re-expression of β-MHC, a marker of myocardial hypertrophy, was found in all CK-deficient hearts. LV remodeling was investigated by MRI in hearts of 7 WT and 10 M/Mito-CK$^{-/-}$ mice 4 weeks postmyocardial infarction (MI). Four weeks post-LAD ligation (MI size ~32%), WT and M/Mito-CK$^{-/-}$ showed a similar degree of cardiac dysfunction, dilatation, and hypertrophy.

Conclusion: Mito-CK$^{-/-}$ and M/Mito-CK$^{-/-}$ mice show significant LV dilatation and marked LV hypertrophy, but LV remodeling post-MI is not aggravated. CK ablation leads to substantial adaptational changes in heart.

Keywords: Energy metabolism; Hypertrophy; NMR; Remodeling; Transgenic animal models

1. Introduction

The failing myocardium is characterized by changes in myocardial energetics, including reduced levels of phosphocreatine, creatine, creatine kinase (CK) activity [1] and flux [2], and, to a lesser degree, of ATP [3]. For the past four decades, scientists have tried to unravel the question whether these alterations in myocardial energetics are a causal mechanism contributing to contractile dysfunction or a coincident or even adaptive phenomenon. A reduction in phosphocreatine, ATP, and in creatine kinase flux, i.e., in the capacity of the creatine kinase shuttle to transport high-energy phosphate bonds from sites of ATP production to sites of ATP utilization, may constitute a pathophysiological phenomenon, while the reduction of total creatine content might potentially be adaptive in keeping free ADP

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levels low and free energy [ΔG_{ATP}] levels high (see Ref. [4] for a review). While some studies have indirectly pointed to the pathophysiological relevance of energetic changes for contractile failure [5–8], the question as to the fundamental role of energetics in heart failure has remained unanswered.

Therefore, when the Wieringa group reported on mice with knockout of M-CK [9] (M-CK$^{-/-}$; 28% of normal CK activity), mitochondrial CK [10] (Mito-CK$^{-/-}$; 70% of normal CK activity), and with combined ("double") M/Mito-CK knockout [11] (4% of normal CK activity), the opportunity arose to test the relevance of the cardiac creatine kinase system in animal models where specific key components of the CK system had been ablated. Consequently, previous studies of these mice have shown that phosphocreatine levels are substantially reduced in both Mito-CK$^{-/-}$ [12] and double CK knockout [13] but remain normal in M-CK$^{-/-}$. Other biochemical and ultrastructural alterations [14–17] have also been described. Surprisingly, previous studies in isolated hearts have so far been unable to detect significant alterations in cardiac function in CK-deficient mice [12,13,15]. However, it is well recognized that while gross phenotypes, such as advanced cardiomyopathy, will become apparent, more subtle indices of murine cardiac performance are difficult to assess in the perfused heart model [18]. Furthermore, many other conditions of energy deficiency have been shown to cause cardiac hypertrophy [5,19–21] and thus an array of adaptational changes that may counteract the deleterious effects of CK ablation.

The purpose of this study was therefore to characterize the in-vivo cardiac phenotype of CK-deficient mouse strains with the most accurate imaging technique available to date, cardiac magnetic resonance imaging (MRI), both under baseline conditions and after development of postmyocardial remodeling. Our hypothesis was that ablation of CK isoenzymes leads to cardiac hypertrophy, impaired function, and aggravation of left ventricular remodeling post-myocardial infarction.

2. Methods

2.1. Animals and experimental protocol

CK-deficient mice were obtained from Dr. Bé Wieringa (University of Nijmegen, The Netherlands). CK-deficient mice had a mixed C57Bl/6–129/Sv background, and wild-type mice were C57Bl/6. Mice of a mean age of 41±2 weeks and equal distribution among both sexes were studied. The genotype of the mice was verified by confirming the complete ablation of the specific CK isoenzymes. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institute of Health (NIH Publication No. 85-23, revised 1996).

In total, 94 mice were investigated. A complete in vivo MR imaging study was performed in 10 wild-type (WT), 6 mitochondrial CK KO (Mito-CK$^{-/-}$), 10 cytosolic CK KO (M-CK$^{-/-}$), and 10 mice with a combined knockout of both CK isoenzymes (M/Mito-CK$^{-/-}$). Thereafter, hearts were harvested for determination of cellular markers of hypertrophy. In addition, 12 WT and 17 M/Mito-CK$^{-/-}$ mice were subjected to left coronary artery ligation as described previously [22]. Mortality of this procedure was identical between groups (WT 42%, M/Mito-CK$^{-/-}$ 41%). MRI investigation on 17 infarcted mice (WT MI n=7, M/Mito-CK$^{-/-}$, n=10) was performed 4 weeks post-MI.

Independently, in six mice per genotype, blood pressure was measured in awake mice with the tail cuff method to rule out the possibility that differences in cardiac function and mass are due to altered blood pressure in CK-deficient mice.

2.2. Cine MRI

Cine MRI was performed on a 7 T-Biospec (Bruker, Germany) using an ECG-triggered fast gradient echo FLASH sequence as previously described in detail [23,24]. Ten to 12 contiguous ventricular short-axis slices of 1-mm thickness were acquired to cover the entire heart. Imaging parameter were as follows: echo time, 1.5 ms; repetition time, 4.3 ms; field of view (30 mm^2); acquisition matrix, 128×128; and slice thickness, 1.0 mm. Analysis of end-systolic and end-diastolic LV volumes and LV mass was done using an operator-interactive threshold technique, and stroke volume and cardiac output were calculated, all as previously described [25]. Myocardial infarct size (MI size) was determined for every slice as the myocardial portion with significant thinning and akinesia during systole.

2.3. Determination of autopsy wet weight and of myocyte cross-sectional area

The blotted wet weight of hearts excised after euthanasia was determined for an additional four to six age-matched mice per genotype. In addition, to further confirm the finding of hypertrophy, we measured myocyte cross-sectional areas in an additional 4 WT and 4 M/Mito-CK$^{-/-}$ mice. Hearts were fixed in formalin, dehydrated, and embedded in paraffin. Slices of 5-μm thickness were produced for 10 short-axis slices covering the entire left ventricle. Slices were stained by hematoxylin/eosin and scanned with 25× magnification using a microscope and a mounted Nikon coolpix digital camera. Sigma Scan pro 5 software was used for planimetry of myocytes. Ten myocytes, which were sliced strictly orthogonally and exhibited a cell nucleus, were segmented for each of the 10 short-axis slices, resulting in a total of 100 myocyte cross-sectional areas per heart.

Table 1
Baseline characteristics and MRI analysis of cardiac function and anatomy in mice of different genotypes

	Wild-type n=10	M-CK$^{-/-}$ n=10	Mito-CK$^{-/-}$ n=6	M/Mito-CK$^{-/-}$ n=10
Total CK activity [% of wild-type]	100	28±3	68±5	<3±1
Body weight [g]	26.2±0.8	26.2±1.1	28.2±1.3	27.4±0.7
Heart rate [beats/min]	435±30	407±19	401±58	471±35
End-diastolic volume [μl]	70.0±3.1	65.8±3.5	92.9±5.3*,†	88.9±5.1*,†
Ejection fraction [%]	63.6±2.6	66.8±3.0	65.2±2.6	62.1±3.3
Stroke volume [μl]	44.5±2.8	43.3±1.9	60.6±4.6*,†	54.4±2.9*,†
Cardiac output [ml/min]	19.3±1.6	17.6±1.2	24.3±2.4	25.6±2.3*,†
Blood pressure [mm Hg]	128.0±2.7	132.8±6.2	100.4±0.3*,†,‡	130.0±4.0

Mean±S.E.M.
* p<0.05 vs. wild-type.
† p<0.05 vs. M-CK$^{-/-}$.
‡ p<0.05 vs. M/Mito-CK$^{-/-}$.

2.4. Cellular markers of hypertrophy

Samples for high-resolution protein gel electrophoresis for MHC analysis were prepared as follows: samples were homogenized in low-salt buffer (20 mmol/L KCl, 2 mmol/L KH_2PO_4, 1 mmol/L EGTA, pH 6.8, 1 mmol/L PMSF, 100 μL *N*,*N*-dimethyl formamide) and were then centrifuged at 5000 rpm for 10 min at 4 °C. Laemmli's buffer was added to each sample. Gels were run as described earlier [26]. Gel samples (0.25 to 1 μg) were loaded in a 3-μL volume onto 15-well gels. The stacking and separating gels (0.75 mm thick) consisted of 4% and 8% acrylamide, respectively; the stacking gels included 5% glycerol. The gels were run at a constant voltage of 200 V for 30 h and were fixed and silver-stained. A gel documentation system (Bio-Rad) was used to scan the stained gels.

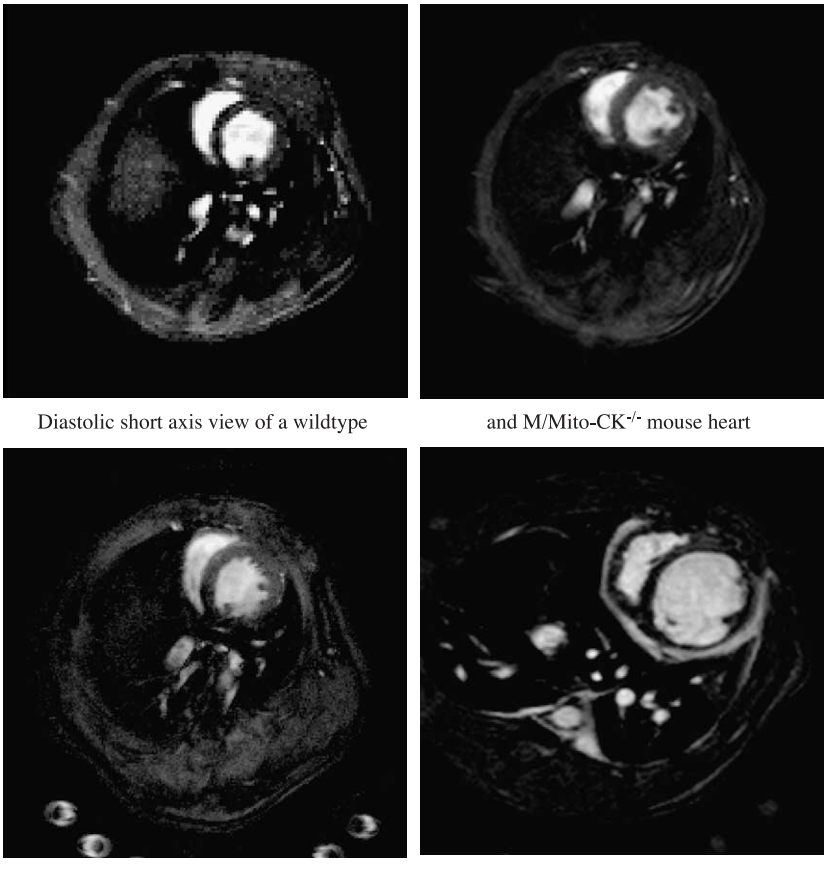


Fig. 1. Representative end-diastolic short-axis frames of a wild-type (left) and an M/Mito-CK$^{-/-}$ mouse (right) are shown in the top row. The higher wall thickness of the CK $^{-/-}$ mouse is apparent. At the bottom row, a short-axis frame of a wild-type mouse heart with (right) and without myocardial infarction is depicted.

2.5. Measurement of blood pressure in the awake mouse

Systolic blood pressure was measured in unanesthetized mice by the tail cuff method (Blood Pressure Monitor BMN-1756, Föhr Medical Instruments, Seeheim, Germany) as previously described [27]. The mice underwent 4 days of training to get accustomed to this procedure. Mean values of five subsequent measurements were calculated.

3. Statistics

Results are expressed as mean±S.E.M. Statistical comparisons among various groups were evaluated by ANOVA, followed by Duncan test to isolate significance of differences between individual means. $P<0.05$ was considered to indicate statistical significance. For comparison of infarcted WT and M/Mito-CK$^{-/-}$ mice, a Student's *t*-test was used.

4. Results

4.1. Magnetic resonance imaging of wild-type and creatine kinase knockout mice

Body weights and heart rates of anaesthetized mice during MRI measurements were similar for all groups (Table 1). Representative examples of a diastolic short-axis view of a WT and a M/Mito-CK$^{-/-}$ mouse are shown in Fig. 1. As summarized in Fig. 2A, we found a marked and highly significant increase in LV mass in Mito-CK$^{-/-}$ (+73%) and M/Mito-CK$^{-/-}$ (+64%) as compared to WT mice. In M-CK$^{-/-}$, LV mass was between that of WT and Mito-CK$^{-/-}$ or M/Mito-CK$^{-/-}$ mice, but the increase did not reach statistical significance. However, LV mass of Mito-CK$^{-/-}$ and of M/Mito-CK$^{-/-}$ mice was significantly higher than LV mass of M-CK$^{-/-}$. Changes in end-diastolic wall thickness were consistent with these differences in LV mass (Fig. 2B). Furthermore, we found significant LV dilatation, i.e., a ~30% increase in LV end-diastolic volume in both Mito-CK$^{-/-}$ and M/Mito-CK$^{-/-}$, but not in M-CK$^{-/-}$ (Table 1). In contrast, LV ejection fraction was normal in all groups, and cardiac output was significantly higher in M/Mito-CK$^{-/-}$ than in WT and M-CK$^{-/-}$. Stroke volumes were also found to be higher in Mito-CK$^{-/-}$ and M/Mito-CK$^{-/-}$ mice.

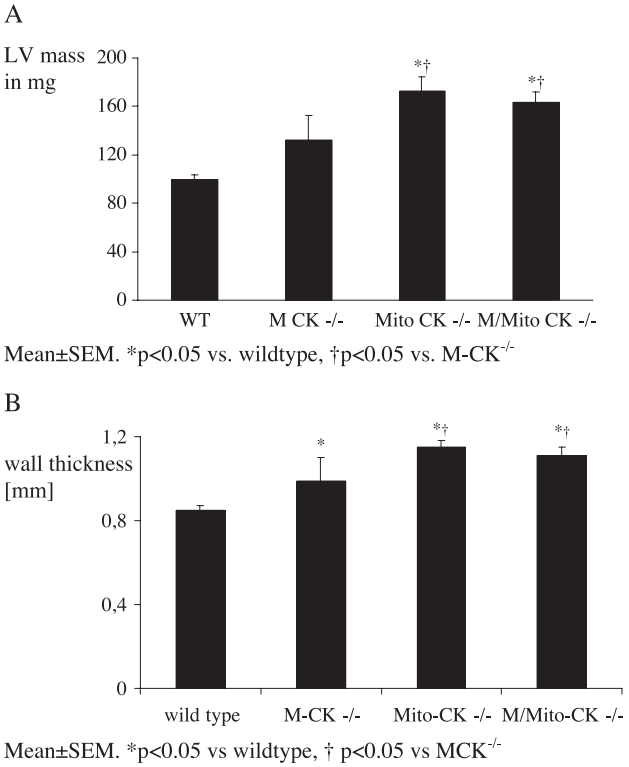


Fig. 2. LV mass (A) and end-diastolic wall thickness (B) in mice of different genotypes.

Table 2
MRI data of wild-type and M/Mito-CK$^{-/-}$ mice 4 weeks after myocardial infarction

	MI wild-type *n*=7	MI M/Mito-CK$^{-/-}$ *n*=10
Body weight [g]	28.1±0.3	25.5±1.1
Heart rate [beats/min]	479±44	421±22
Infarct size [%]	32±6	31±2
Left ventricular mass [mg]	159.1±14.3	163.1±13.5
End-diastolic volume [μl]	186.2±39.9	143.5±14.5
Ejection fraction [%]	37±6	40±5
Stroke volume [μl]	54.3±14.9	53.0±8.4
Cardiac output [ml/min]	26.0±3.2	22.3±1.8
End-diastolic wall thickness [mm]	1.0±0.07	1.1±0.07

Mean±S.E.M.

4.2. Magnetic resonance imaging after left ventricular remodeling

Infarct sizes of WT and M/Mito-CK$^{-/-}$ mice were similar (32% vs. 31%; Table 2). In infarcted WT mice, coronary ligation induced the characteristic changes of LV remodeling after 4 weeks of chronic myocardial infarction. Specifically, we found a 60% increase in LV mass, a 166% increase in left ventricular end-diastolic volume due to chamber dilatation, and a markedly reduced ejection fraction (Table 2). Interestingly, compared to wild-type mice, ejection fraction and end-diastolic volume of chronically infarcted M/Mito-CK$^{-/-}$ mice were not different (Table 2). Furthermore, while noninfarcted M/Mito-CK$^{-/-}$ mice showed substantial LV hypertrophy, myocardial infarction did not induce any additional hypertrophy in these animals, and LV mass was similar in wild-type and M/Mito-CK$^{-/-}$ mice 4 weeks after infarction.

4.3. Blood pressure

Systolic blood pressure, independently measured on subsequent days in five conscious mice per genotype, was indistinguishable between WT, M-CK$^{-/-}$, and

M/Mito-CK$^{-/-}$ mice (Table 1). Mito-CK$^{-/-}$ were found to have a lower blood pressure compared to wild-type.

4.4. Determination of autopsy LV weight and of myocyte cross-sectional area

Measurement of LV mass by MRI had previously been extensively validated by our group [25,28]. To confirm the MRI results, LV weight was determined at autopsy in independent groups of age-matched mice. LV weight was 100±3 mg in wild-type (n=12), 119±3 mg in M-CK$^{-/-}$ (n=7), 135±12 mg in Mito-CK$^{-/-}$ (n=7, p<0.05 vs. WT), and 163±9 mg in M/Mito-CK$^{-/-}$ (n=10, p<0.05 vs. all).

To further confirm cardiac hypertrophy in CK knockout mice, myocyte cross-sectional areas were measured in four wild-type and four M/Mito-CK$^{-/-}$ mice. Myocyte sizes were 231.1±5.4 μm^2 in wild-type and 301.0±8.0 μm^2 in M/Mito-CK$^{-/-}$ hearts (p<0.05 vs. WT).

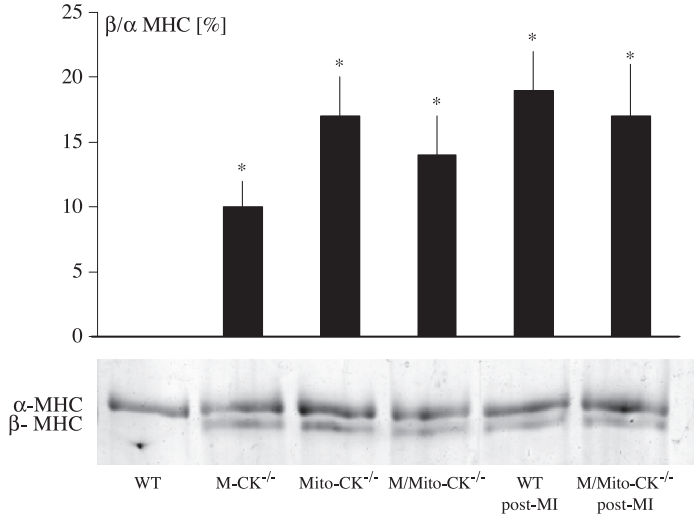
4.5. Myosin heavy chain analysis

Because the relative proportions of the two forms of the MHC have been shown to be an independent indicator of the molecular response of the sarcomere to various external and internal stimuli, we determined the MHC protein isoform content. As expected, in WT hearts, only α-MHC was detectable. However, 4 weeks after chronic myocardial infarction, a significant reexpression of β-MHC, as a marker of cardiac hypertrophy, was detectable in the infarcted WT hearts (25±4% β-MHC of total MHC). In contrast, in all CK-deficient hearts, with or without myocardial infarction, a reexpression of a significant amount of β-MHC was found (Fig. 3). The amount of β-MHC expression ranged between 10±2% (for M-CK$^{-/-}$) and 17±3% (for Mito-CK$^{-/-}$) of total MHC. The effects of CK knockout and chronic infarction on β-MHC expression were not additive, similar to our observations for LV mass.

5. Discussion

5.1. Creatine kinase-deficient hearts show cardiac hypertrophy and dilatation

Evolution has preserved creatine kinase at high levels in hearts of all mammalian species. Therefore, when initial reports on creatine kinase-deficient mice had shown that isolated perfused heart function appeared to be unaltered [12,13,15], the question arose whether a highly abundant protein, such as creatine kinase, can indeed be ablated in heart without significant functional consequences. In this study, we clearly demonstrate that ablation of the mito-CK gene, but not of the M-CK gene, leads to a striking phenotype: our major finding is the presence of substantial LV hypertrophy in Mito-CK- and M/Mito-CK-deficient mice. We show that deleting the mito-CK isoenzyme (Mito-CK$^{-/-}$ and M/Mito-CK$^{-/-}$) leads to an approximately 70% increase in LV weight compared to age- and body weight-matched wild-type animals, and this finding was confirmed by autopsy. The two previous studies reporting on cardiac mass in M/Mito-CK$^{-/-}$ showed that at 34 weeks of age LV mass increased from 138 in wild-type to 171 mg in M/Mito-CK$^{-/-}$ (a 24% increase) [29], and that the heart weight/body weight ratio increased from 5.0 to 6.0 (a 20% increase) at 13 weeks of age [14]. Our results were obtained at 41 weeks of age, and it is, thus, likely that cardiac hypertrophy due to CK deficiency is progressive with increasing age and may be present to a lesser degree when hearts are studied at younger age. Because the current study provides data at only one time point, future longitudinal studies are



Mean±SEM. *p<0.05 vs. wildtype sham.

Fig. 3. Original photograph of α- and β-MHC (myosin heavy chain) distribution in the myocardium of the different genotypes. Infarcted mice and CK-deficient mice display expression of β-MHC as a marker of myocardial hypertrophy, whereas WT sham mice have no expression of β-MHC.

necessary to define the full-time course of the development of LV changes in M/Mito-CK$^{-/-}$ hearts. However, other reasons for underestimation of LV mass in previous studies may be that LV weight was obtained after isolated perfused heart experiments, where edema formation leads to changes in heart weight [12,13]. Clearly, in vivo MRI is the most accurate technique for quantification of cardiac mass both in humans [30] and in mice [28].

One potential mechanism that might lead to the development of LV hypertrophy would be changes in blood pressure regulation leading to chronically increased afterload. Therefore, we measured blood pressure in unrestrained awake mice and were able to rule out that cardiac hypertrophy seen in M/Mito-CK$^{-/-}$ and in Mito-CK$^{-/-}$ mice is due to this mechanism. The reasons for reduced blood pressure in Mito-CK$^{-/-}$ mice are unclear from this study and remain to be further elucidated, but this effect would tend to decrease LV mass, while a significant increase was observed.

To further confirm the presence of cardiomyocyte hypertrophy, we also measured myocyte cross-sectional areas of wild-type and M/Mito-CK$^{-/-}$ hearts, and found that M/Mito-CK$^{-/-}$ hearts exhibited a significant increase (30%) in myocyte size. These results are in line with electron microscopy reports by Kaasik et al. [14], showing ultrastructural reorganisation of cardiac muscle with thinning of myofilament bundles and abundance of mitochondria.

Finally, a third line of independent evidence for a hypertrophic response to CK deficiency is provided by the biochemical assessment of the molecular response of the sarcomere. The relative proportion of the two forms of the MHC has been shown to be affected by a wide variety of pathological and physiological stimuli [31]. Work from several groups has shown that in the rodent heart, the MHC phenotype switches from the β-MHC isoform to the α-MHC isoform during maturation, and vice versa under increased hemodynamic load or disease states [32]. This isoform switch is associated with alterations in the functional and energetic behavior of the contractile apparatus. α-MHC leads to a higher maximal shortening velocity but lower economy in tension development. In contrast, high amounts of β-MHC decrease maximal shortening velocity and increase economy of contraction and may preserve function in overloaded cardiomyocytes. A switch to β-MHC is accepted as a marker of myocardial hypertrophy [33]. Interestingly, in our study, not only hearts with the greatest extent of hypertrophy, i.e., Mito-CK$^{-/-}$ and M/Mito-CK$^{-/-}$, showed a substantial increase in the β-MHC but also M-CK$^{-/-}$ mice, which showed a borderline increase in cardiac mass. This may suggest that the MHC ratio is an early and sensitive indicator of the process of hypertrophy development. It is also noteworthy that the extent of β-MHC reexpression induced by CK deficiency was similar to the reexpression measured in remodeled wild-type hearts 4 weeks after myocardial infarction, but effects of CK deficiency and of chronic remodeling were not additive.

Consistent with previous reports in isolated hearts [12,13,15], we found LV performance to be well preserved, as estimated by ejection fraction and cardiac output. However, we report for the first time that both Mito-CK$^{-/-}$ and M/Mito-CK$^{-/-}$CK-deficient hearts, but not M-CK$^{-/-}$, showed a significant degree of left ventricular dilatation, and end-diastolic volumes were increased by approximately 30%. This finding would escape detection in isolated heart models where end-diastolic pressure and volume are typically set to an artificial value (e.g., EDP=4 mm Hg). Interestingly, in the only study assessing LV function in M/Mito-CK$^{-/-}$ mice in vivo [34], using echocardiography, although the point was not made or discussed in the manuscript, end-diastolic diameters of M/Mito-CK$^{-/-}$ mice were actually increased from 3.39 to 3.70 mm. Recognizing the limitations of this calculation, using Teichholz' formula [35] to calculate LV volumes from M-mode diameters, this would correspond to an increase of LV volume by 38%, which is similar to what we observed in this study. Echocardiography did not show an increase in LV wall thickness in Mito-CK$^{-/-}$ hearts, and this discrepancy is likely due to the fact that MRI is much more sensitive in detecting changes in LV wall thickness in the miniature mouse heart. Our finding clearly indicates that CK-deficient hearts are in a compensated stage of cardiac hypertrophy, with maintained ejection fraction, increased LV volume, and absence of any clinical signs of cardiac failure, such as ascites or pleural effusion.

6. Hypertrophy and cardiac energy metabolism

Previous work has shown that deleting Mito and M/Mito-CK isoenzymes leads to a significant decrease in the PCr/ATP ratio, as well as to an increase in the free ADP concentration and to a decrease in the free energy available from ATP hydrolysis [ΔG_{ATP}] [12,13,15]. The present report demonstrates that a net effect of these energetic changes is the development of substantial LV hypertrophy. In fact, the phenomenon of compromised energy metabolism leading to cardiac hypertrophy is a recurrent theme. When a creatine analoge, β-guanidinoproprionate, is fed to deplete creatine and, subsequently, phosphocreatine, the substrates of the creatine kinase reaction, a significant increase in LV mass was observed [36]. Ablation of the insulin-dependent glucose transporter GLUT4 leads to substantial cardiac hypertrophy [19]. Furthermore, in mice with knockout of one of the enzymes essential for creatine synthesis (GAMT), we have recently described a 15% increase in the heart weight/body weight ratio [37]. In fact, an energy compromised state has recently been proposed as the common pathophysiological mechanism causing hypertrophy in patients with hypertrophic cardiomyopathy due to numerous mutations [38]. While we describe cardiac hypertrophy in response to CK knockout, the various adaptations leading to the development of macroscopic and microscopic hypertrophy under these circumstances will

have to be elucidated in future studies. In the presence of compromised ATP transfer to sites of ATP utilization due to lack of CK shuttle components, the main obvious beneficial effect of hypertrophy development in CK-deficient hearts would be to reduce LV wall stress according to Laplace's law and thus reduce ATP demand and utilization. Other lines of investigations will be to study the signaling mechanisms involved in initiating a hypertrophic response in CK-deficient mice. Because cardiac energy metabolism is intimately linked to the intracellular calcium homeostasis [39], alterations in calcium metabolism are one likely candidate for this. Several previous reports have already shown significant disturbances in calcium homeostasis of CK-deficient mice [11,29,34]. An alternative mechanism may be related to K_{ATP} channel function; as recently demonstrated, knockout of K_{ATP} channels leads to development of left ventricular hypertrophy. Functional coupling between K_{ATP} channels and creatine kinase has been demonstrated, and CK-M$^{-/-}$ mice were shown to have impaired K_{ATP} channel function [40].

7. Chronic myocardial infarction in CK-deficient hearts

We also showed that hearts lacking almost their entire CK activity did not exhibit accelerated or adverse remodeling postmyocardial infarction, and neither a higher acute mortality nor a larger reduction of ejection fraction or increase in LV chamber size was detectable. While this finding may at first sight be surprising, other groups have shown that preexisting hypertrophy protects the heart from the consequences of LV remodeling postmyocardial infarction. Litwin et al. [41] showed that induction of myocardial hypertrophy with an inhibitor of long-chain fatty acid oxidation prevented LV dilatation and improved systolic function after chronic coronary ligation. Similarly, Zdrojewski et al. [42] demonstrated that preexisting LV hypertrophy in spontaneous hypertensive rats prevented LV dilatation and maintained systolic function in infarcted hearts, inspite of increased afterload conditions. The most likely explanation for this phenomenon is that increased wall thickness leads to reduced wall stress and energy demand of residual intact myocardium post-MI, thereby attenuating the deleterious consequences of LV remodeling. Therefore, it is possible that the adverse effects of CK ablation and the beneficial effects of preexisting hypertrophy counterbalance themselves in M/Mito-CK$^{-/-}$ hearts.

7.1. Role of creatine kinase in heart failure

What do the present results tell us about the role of creatine kinase changes in heart failure? Unfortunately, CK knockout mice have so far not allowed us to unequivocally unravel this unresolved question. What is clear from the present findings is that CK ablation can only be tolerated in the heart if major adaptational changes occur leading to massive LV hypertrophy. However, in heart failure, changes of CK activity and flux are much less pronounced than those in the CK-knockout model, yet they occur in concert with a variety of changes in other, e.g., calcium regulatory systems. Furthermore, in the CK-knockout model, major adaptations and ultrastructural reorganisation can occur during embryonal development, but this is not so in the failing myocardium. Thus, while our current study provides proof of principle of the necessity of CK for a normal cardiac phenotype, unraveling the role of energetics in heart failure will require additional models, such as, e.g., conditional knockout of CK in adult mice.

8. Limitations

In the original publications on the generation of the three different mouse CK knockout lines, it was described that mutant mice had a mixed genetic background of C57BL/6 (from blastocysts) and 129/Sv (embryonic stem cells) [9,11]. To our knowledge, thereafter all subsequent reports that have further investigated the phenotype of the CK-deficient mice, including this study, used age-, sex-, and weight-matched corresponding C57BL/6 mice as wild-type. Although the genetic background of wild-type mice is undoubtedly of substantial importance, it is unlikely that a ~70% increase in LV mass can be explained by the genetic background. More importantly, for almost all parameters measured, M-CK$^{-/-}$ mice, which have a genetic background close to that of the other CK knockout strains, were similar to wild-type mice, and both wild-type and M-CK$^{-/-}$ were significantly different from Mito-CK$^{-/-}$ and M/Mito-CK$^{-/-}$. Thus, it is highly unlikely that observed effects were due to differences in genetic backgrounds. However, for future investigations it will be helpful to generate congenic CK-knockout mice on a pure inbred background.

We did not perform stress studies in our mice, such as, e.g., dobutamine studies. Crozatier et al. [34] have shown, using echocardiography, that the inotropic response to isoprenaline is substantially blunted in M/Mito-CK$^{-/-}$ mice.

9. Conclusion

Our study shows that ablation of the M-creatine kinase gene does not, but ablation of the mitochondrial creatine kinase gene does, have major functional consequences in the heart. Mito-CK$^{-/-}$ and M/Mito-CK$^{-/-}$ mice develop substantial cardiac hypertrophy and left ventricular dilatation. However, CK-deficient hearts do not develop adverse left ventricular remodeling post-MI, probably due to the protective effect of preexisting hypertrophy.

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References

[1] Ingwall JS, Kramer MF, Fifer MA, Lorell BH, Shemin R, Grossman W, et al. The creatine kinase system in normal and diseased human myocardium. N Engl J Med 1985;313(17):1050–4.
[2] Neubauer S, Horn M, Naumann A, Tian R, Hu K, Laser M, et al. Impairment of energy metabolism in intact residual myocardium of rat hearts with chronic myocardial infarction. J Clin Invest 1995;95(3): 1092–100.
[3] Starling RC, Hammer DF, Altschuld RA. Human myocardial ATP content and in vivo contractile function. Mol Cell Biochem 1998;180(1–2):171–7.
[4] Ingwall JS. ATP and the heart: basic science for the cardiologist. Kluwer Academic Publisher.
[5] Horn M, Remkes H, Stromer H, Dienesch C, Neubauer S. Chronic phosphocreatine depletion by the creatine analogue beta- guanidinopropionate is associated with increased mortality and loss of ATP in rats after myocardial infarction. Circulation 2001; 104(15):1844–9.
[6] Liao R, Nascimben L, Friedrich J, Gwathmey JK, Ingwall JS. Decreased energy reserve in an animal model of dilated cardiomyopathy. Relationship to contractile performance. Circ Res 1996;78(5): 893–902.
[7] Nascimben L, Friedrich J, Liao R, Pauletto P, Pessina AC, Ingwall JS. Enalapril treatment increases cardiac performance and energy reserve via the creatine kinase reaction in myocardium of Syrian myopathic hamsters with advanced heart failure. Circulation 1995; 91(6):1824–33.
[8] Tian R, Ingwall JS. Energetic basis for reduced contractile reserve in isolated rat hearts. Am J Physiol 1996;270(4 Pt 2):H1207–16.
[9] van Deursen J, Heerschap A, Oerlemans F, Ruitenbeek W, Jap P, ter Laak H, et al. Skeletal muscles of mice deficient in muscle creatine kinase lack burst activity. Cell 1993;74(4):621–31.
[10] Steeghs K, Heerschap A, de Haan A, Ruitenbeek W, Oerlemans F, van Deursen J, et al. Use of gene targeting for compromising energy homeostasis in neuro- muscular tissues: the role of sarcomeric mitochondrial creatine kinase. J Neurosci Methods 1997;71(1):29–41.
[11] Steeghs K, Benders A, Oerlemans F, de Haan A, Heerschap A, Ruitenbeek W, et al. Altered Ca2+ responses in muscles with combined mitochondrial and cytosolic creatine kinase deficiencies. Cell 1997;89(1):93–103.
[12] Spindler M, Niebler R, Remkes H, Horn M, Lanz T, Neubauer S. Mitochondrial creatine kinase is critically necessary for normal myocardial high-energy phosphate metabolism. Am J Physiol, Heart Circ Physiol 2002;283(2):H680–7.
[13] Saupe KW, Spindler M, Tian R, Ingwall JS. Impaired cardiac energetics in mice lacking muscle-specific isoenzymes of creatine kinase. Circ Res 1998;82(8):898–907.
[14] Kaasik A, Veksler V, Boehm E, Novotova M, Minajeva A, Ventura-Clapier R. Energetic crosstalk between organelles: architectural integration of energy production and utilization. Circ Res 2001; 89(2):153–9.
[15] Saupe KW, Spindler M, Hopkins JC, Shen W, Ingwall JS. Kinetic, thermodynamic, and developmental consequences of deleting creatine kinase isoenzymes from the heart. Reaction kinetics of the creatine kinase isoenzymes in the intact heart. J Biol Chem 2000;275(26): 19742–6.
[16] Veksler VI, Kuznetsov AV, Anflous K, Mateo P, van Deursen J, Wieringa B, et al. Muscle creatine kinase-deficient mice: II. Cardiac and skeletal muscles exhibit tissue-specific adaptation of the mitochondrial function. J Biol Chem 1995;270(34):19921–9.
[17] Ventura-Clapier R, Kuznetsov AV, d'Albis A, van Deursen J, Wieringa B, Veksler VI. Muscle creatine kinase-deficient mice: I. Alterations in myofibrillar function. J Biol Chem 1995;270(34):19914–20.
[18] Kass DA, Hare JM, Georgakopoulos D. Murine cardiac function: a cautionary tail. Circ Res 1998;82(4):519–22.
[19] Weiss RG, Chatham JC, Georgakopolous D, Charron MJ, Wallimann T, Kay L, et al. An increase in the myocardial PCr/ATP ratio in GLUT4 null mice. FASEB J 2002;16(6):613–5.
[20] Tian R, Musi N, D'Agostino J, Hirshman MF, Goodyear LJ. Increased adenosine monophosphate-activated protein kinase activity in rat hearts with pressure-overload hypertrophy. Circulation 2001;104(14): 1664–9.
[21] Tian R. Transcriptional regulation of energy substrate metabolism in normal and hypertrophied heart. Curr Hypertens Rep 2003;5(6):454–8.
[22] Frantz S, Hu K, Widder J, Bayer B, Witzel CC, Schmidt I, et al. Peroxisome proliferator activated-receptor agonism and left ventricular remodeling in mice with chronic myocardial infarction. Br J Pharmacol 2004;141(1):9–14.
[23] Haase A, Matthaei D, Hanicke W, Frahm J. Dynamic digital subtraction imaging using fast low-angle shot MR movie sequence. Radiology 1986;160(2):537–41.
[24] Ruff J, Wiesmann F, Hiller KH, Voll S, von Kienlin M, Bauer WR, et al. Magnetic resonance microimaging for noninvasive quantification of myocardial function and mass in the mouse. Magn Reson Med 1998; 40(1):43–8.
[25] Nahrendorf M, Wiesmann F, Hiller KH, Han H, Hu K, Waller C, et al. In vivo assessment of cardiac remodeling after myocardial infarction in rats by cine-magnetic resonance imaging. J Cardiovasc Magn Reson 2000;2(3):171–80.
[26] Cummins P, Lambert SJ. Myosin transitions in the bovine and human heart. A developmental and anatomical study of heavy and light chain subunits in the atrium and ventricle. Circ Res 1986;58(6):846–58.
[27] Quaschning T, Ruschitzka F, Stallmach T, Shaw S, Morawietz H, Goettsch W, et al. Erythropoietin-induced excessive erythrocytosis activates the tissue endothelin system in mice. FASEB J 2003;17(2): 259–61.
[28] Wiesmann F, Ruff J, Hiller KH, Rommel E, Haase A, Neubauer S. Developmental changes of cardiac function and mass assessed with MRI in neonatal, juvenile, and adult mice. Am J Physiol, Heart Circ Physiol 2000;278(2):H652–7.
[29] Boehm E, Ventura-Clapier R, Mateo P, Lechene P, Veksler V. 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 2000;32(6):891–902.
[30] Bellenger NG, Davies LC, Francis JM, Coats AJ, Pennell DJ. Reduction in sample size for studies of remodeling in heart failure by the use of cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2000;2(4):271–8.
[31] Ritter O, Neyses L. The molecular basis of myocardial hypertrophy and heart failure. Trends Mol Med 2003;9(7):313–21.
[32] Schwartz K, Chassagne C, Boheler KR. The molecular biology of heart failure. J Am Coll Cardiol 1993;22(4 Suppl. A):30A–3A.
[33] Small EM, Krieg PA. Molecular regulation of cardiac chamber-specific gene expression. Trends Cardiovasc Med 2004;14(1):13–8.
[34] Crozatier B, Badoual T, Boehm E, Ennezat PV, Guenoun T, Su J, et al. Role of creatine kinase in cardiac excitation-contraction coupling: studies in creatine kinase-deficient mice. FASEB J 2002;16(7):653–60.
[35] Teichholz LE, Cohen MV, Sonnenblick EH, Gorlin R. Study of left ventricular geometry and function by B-scan ultrasonography in patients with and without asynergy. N Engl J Med 1974;291(23):1220–6.
[36] Mekhfi H, Hoerter J, Lauer C, Wisnewsky C, Schwartz K, Ventura-Clapier R. Myocardial adaptation to creatine deficiency in rats fed

with beta-guanidinopropionic acid, a creatine analogue. Am J Physiol 1990;258(4 Pt 2):H1151–8.
[37] Schmidt A, Marescau B, Boehm EA, Renema WK, Peco R, Das A, et al. Severely altered guanidino compound levels, disturbed body weight homeostasis, and impaired fertility in a mouse model of guanidinoacetate *N*-methyltransferase (GAMT) deficiency. Hum Mol Genet 2004;13(9):905–21.
[38] Ashrafian H, Redwood C, Blair E, Watkins H. Hypertrophic cardiomyopathy: a paradigm for myocardial energy depletion. Trends Genet 2003;19(5):263–8.
[39] Tian R, Halow JM, Meyer M, Dillmann WH, Figueredo VM, Ingwall JS, et al. Thermodynamic limitation for Ca2+ handling contributes to decreased contractile reserve in rat hearts. Am J Physiol 1998;275(6 Pt 2):H2064–71.
[40] Abraham MR, Selivanov VA, Hodgson DM, Pucar D, Zingman LV, Wieringa B, et al. Coupling of cell energetics with membrane metabolic sensing. Integrative signaling through creatine kinase phosphotransfer disrupted by M-CK gene knock-out. J Biol Chem 2002;277(27):24427–34.
[41] Litwin SE, Raya TE, Anderson PG, Litwin CM, Bressler R, Goldman S. Induction of myocardial hypertrophy after coronary ligation in rats decreases ventricular dilatation and improves systolic function. Circulation 1991;84(4):1819–27.
[42] Zdrojewski T, Gaudron P, Whittaker P, Poelzl S, Schiemann J, Hu K, et al. Ventricular remodeling after myocardial infarction and effects of ACE inhibition on hemodynamics and scar formation in SHR. Cardiovasc Pathol 2002;11(2):88–93.