Chronic high-dose creatine feeding does not attenuate left ventricular remodeling in rat hearts post-myocardial infarction

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Abstract

Objective: In heart failure, cardiac energy metabolism is compromised. The failing myocardium is characterized by reduced contents of both phosphorylated (phosphocreatine) and non-phosphorylated (free) creatine content as well as decreased energy reserve via creatine kinase (creatine kinase reaction velocity). These changes may contribute to cardiac dysfunction. The purpose of the present study was to determine whether chronic feeding with high-dose dietary creatine prevents the derangement of energy metabolism and the development of left ventricular remodeling in a rat model of heart failure, i.e. post-myocardial infarction (MI).

Methods and results: Rats were subjected to sham operation or left coronary artery ligation. Surviving rats were fed with 0% (untreated) or 3% creatine (related to weight of diet) for 8 weeks. Creatine feeding increased serum creatine levels significantly |2-fold. Thereafter, hearts were isolated, perfused and left ventricular pressure±volume curves obtained. Steady state and dynamic (CK reaction velocity) high-energy phosphate metabolism was determined with 31P NMR spectroscopy. In both MI groups (treated n=8, untreated n=7), pressure–volume curves were shifted right- and downward compared to both sham groups (treated n=5, untreated n=7), i.e. creatine had no effect on left ventricular remodeling. Likewise, similar reductions of phosphocreatine, free creatine and creatine kinase reaction velocity (untreated sham 12.0±0.7 mmol/l/s; untreated MI 7.8±0.7*; treated sham 13.6±1.0; treated MI 7.2±1.1*; * p<0.025 sham vs. MI) were found in both MI groups.

Conclusions: Chronic creatine feeding of post-MI rats is ineffective in preventing the functional and energetic derangements occurring post-MI. Inspite of increased serum creatine levels, neither the normal nor the failing heart accumulates additional creatine.

Keywords: Creatine; 31P NMR spectroscopy; Heart failure; ATP; Phosphocreatine; Isolated rat heart

1. Introduction

Both experimental [1–3] and clinical studies [4–7] have shown that the hypertrophied and failing myocardium is characterized by a depletion of creatine compounds, i.e. both phosphorylated (phosphocreatine) and unphosphorylated (‘free’) creatine are decreased. The reduction of phosphocreatine is largely responsible for the substantial decrease of energy reserve via creatine kinase (creatine kinase reaction velocity, or ‘flux’) in the failing heart [1,2,8]. Although direct proof has been difficult, it is well conceivable that changes in creatine metabolism and creatine kinase (CK) kinetics contribute to contractile dysfunction (see Ref. [9] for a review). Compounds of proven therapeutic benefit in heart failure such as beta-receptor-blockers [10] or angiotensin-converting enzyme inhibitors [2,11] were shown to increase (phospho-) creatine content and CK reaction velocity in experimental heart failure models in parallel with their beneficial functional effects, but these compounds do not primarily act on energy metabolism. Thus, it would be interesting to study interventions that primarily and specifically increase creatine content and, therefore, improve CK kinetics in heart failure, and to test for their possible beneficial

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functional effects. Cardiomyocytes do not synthesize creatine but accumulate the compound against a large concentration gradient through the action of the specific Na\(^+\)-creatine cotransporter protein [12]. Studies in athletes have shown that creatine content of skeletal muscle can be increased 15–20% by chronically supplying high dosages of creatine with the diet, and that skeletal muscle work output increases in parallel [13], although in rat, this has not been observed [15].

Based on these observations, purpose of the present work was to study the effects of chronically supplying high dietary dosages of creatine on myocardial creatine and phosphocreatine content and on cardiac structure and function in a clinically highly relevant model of heart failure, i.e. the rat heart post-myocardial infarction (MI). If successful, oral creatine might have been a novel approach to the treatment of chronic heart failure. However, our results indicate that, inspite of substantially increased extracellular creatine concentrations, neither the normal nor the failing heart increases its creatine content.

2. Methods

2.1. Animals and experimental myocardial infarction

Infarcts or sham operations were carried out in 12-weeks old Wistar rats as previously described [1,16]. The left coronary artery was ligated after left thoracotomy under ether anesthesia. Mortality rate of infarcted rats for the first 24 h after the operation was 40–50%. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1985).

2.2. Isolated heart preparation

Rats were re-anesthetized by injecting 50 mg pentobarbital sodium intraperitoneally. After thoracotomy, the heart was rapidly excised and immersed in ice-cold buffer. The aorta was dissected free, and mounted onto a cannula attached to a perfusion apparatus, as previously described [17]. Retrograde perfusion of the heart was started in the Langendorff mode at a constant temperature of 37°C and a constant coronary perfusion pressure of 100 mmHg. For perfusion, phosphate-free Krebs–Henseleit buffer was used as described [17]. Coronary flow was measured by an ultrasonic flow probe (Transonic Systems Inc., Ithaca, NY). As previously shown, the perfusion system allowed maintenance of hearts in a steady state for at least 90 min with changes of less than 5% for all mechanical and metabolic parameters [17]. For measurement of cardiac performance, a water-filled latex balloon was inserted into the left ventricle. The balloon was connected to a Statham P23Db pressure transducer (Gould Instruments, Glen Burnie, MD) with a small-bore polyethylene tubing for continuous measurement of left ventricular pressure and heart rate on a MacLab system (version 3.5, AD instruments, Castle Hill, Australia). Left ventricular pressure–volume curves were obtained by increasing balloon volume in a stepwise manner (delta 0.05 ml) until maximum LVDP was reached or until LVEDP exceeded 50 mmHg. Thereafter, LVEDP was set to 10 mmHg for the duration of the NMR measurements. At the end of the protocol, hearts were taken off the cannula, and the right ventricle was cut off and rapidly frozen. The entire left ventricle was fixed in formalin as previously described for histologic determination of infarct size.

2.3. Determination of infarct size

Infarct size was determined by a previously described technique [1]. The left ventricle was embedded in paraffin, and 20-μm sections were cut serially from apex to base of the heart. Sections were stained with Picrosirius Red and were mounted on glass plates, scanned (Nikon EF scanner, Nikon, Japan) and planimetry was performed with the NIH Image software (version 1.59, National Institutes of Health). Lengths of scar and non-infarcted muscle for both endocardial and epicardial surfaces were determined for each section. The ratio of the lengths of scar and surface circumferences defined the infarct size for endo- and epicardial surfaces, respectively. Final infarct size was determined as the average of endo- and epicardial surfaces and is given in percent. All hearts with an infarct size of less than 25% were excluded from the analysis.

2.4. 31P NMR spectroscopy

The perfused hearts were placed into a 20-mm NMR sample tube and inserted into a probe seated in the bore of a superconducting super-wide-bore (150 mm) 7.05 Tesla magnet (Bruker, Rheinstetten, FRG) as previously described [1,11,18]. Hearts were bathed in their own perfusate. An Aspect 3000 computer (Bruker, Rheinstetten, FRG) was used in the pulsed Fourier transform mode to generate 31P NMR spectra at 121.50 MHz. A 14-channel Shim Unit served to homogenize the magnetic field. Single (‘one pulse’) spectra were accumulated over 5-min periods, averaging data from 152 free induction decays obtained using a pulse time of 37.6 ms, a pulse angle of 45° and an interpulse delay of 1.93 s. The resonance areas corresponding to ATP, phosphocreatine, inorganic phosphate, monophosphate esters and NAD, which are proportional to the number of phosphorus atoms of the respective compound, were measured using the NMR1 software package (Tripos, St. Louis, MO) and were corrected for partial saturation. In each heart, the area of the [γ-P]ATP resonance of the first spectrum obtained under control conditions was arbitrarily set to 100% and used as the reference value for all resonances in the sequence of 31P.
NMR spectra obtained for the protocol. Absolute ATP concentrations were previously determined for sham hearts as 10.8±0.8 mmol/l, for residual intact left ventricular tissue of MI hearts as 10.6±0.8 mmol/l [1]. Since the protocol (histologic determination of left ventricular infarct size, cutoff of right ventricle for creatine analysis within ~20 s) did not allow absolute ATP quantification by HPLC, ATP concentrations for sham and MI hearts were assumed to be the same as for the previous study [1]. In addition, it was assumed that chronic creatine feeding does not increase ATP above normal values and does not decrease ATP in sham or MI hearts, both assumptions very likely to be correct: ATP cannot increase above normal levels, since mitochondrial ATP production is subject to close feedback inhibition by ATP; chronic creatine feeding does not decrease myocardial ATP content, as was previously shown [15]. Intracellular pH (pH\textsubscript{i}) was measured by comparing the chemical shift between inorganic phosphate and phosphocreatine with values obtained from a standard curve [19].

2.5. \textsuperscript{31}P NMR magnetization transfer measurements of creatine kinase kinetics

For magnetization transfer experiments each broadband pulse was preceded by a low-power, narrowband pulse at the resonance frequency of [γ-P]ATP for 0 or 3.6 s as previously described [15]. Recycle times for each scan were kept constant at 5.0 s. A saturation transfer experiment was acquired in 12 min. Stability of the preparation was assessed by comparing one-pulse spectra obtained before and after each magnetization transfer experiment. Magnetization transfer measurements of the forward CK reaction, phosphocreatine→[γ-P]ATP, were analyzed according to the two-site chemical exchange model of Forsen and Hoffman [20], providing estimates of the pseudo first-order rate constant (K\textsubscript{eq}). Multiplying the rate constant by substrate concentration yielded reaction velocity [19].

2.6. Measurement of creatine content

At the end of the experiment, the right ventricular free wall was cut off and was rapidly frozen in liquid nitrogen for determination of total creatine content by high pressure liquid chromatography (HPLC) as previously described [15,21]. Free creatine was then calculated for each heart as total creatine minus phosphocreatine. Non-collagen protein was measured by the method of Lowry et al. [22]. In analogy to high-energy phosphate concentrations, free creatine concentrations were expressed in mmol/l, assuming that 50% of wet weight represents intracellular H\textsubscript{2}O [23]. Blood was centrifuged, and serum creatine concentrations were measured by HPLC as previously described [15], and were expressed in mmol/l.

2.7. Cytosolic phosphorylation potential

The cytosolic phosphorylation potential (PP; M\textsuperscript{−1}) was calculated as described [24] as:

\[
PP = \frac{ATP}{ADP \times \text{inorganic phosphate}}
\]

where ADP (μmol/l) is

\[
(\text{ATP} \times \text{creatine}) \times H^+ \times K_{eq}.
\]

H\textsuperscript{+} is the intracellular hydrogen ion concentration, and K\textsubscript{eq} is the equilibrium constant of the creatine kinase reaction (1.66×10\textsuperscript{−9} M\textsuperscript{−1}) [11].

2.8. Experimental groups and protocols

Four groups of rats were studied: Sham operated untreated (Sh; n = 7), infarcted untreated (MI; n = 7), sham operated treated (Sh+Cr; n = 5) and infarcted treated (MI+Cr; n = 8). After sham operation or MI, rats were randomized to receive creatine-free chow (fish protein 2.5. Deisenhofen, FRG) was added (% refers to the total weight of chow) for a period of 8 weeks. We had previously shown that this dose of creatine achieves a maximum increase in serum creatine levels without reducing body weight [15]. Food intake was similar (32.4±0.5 g/day) for all four groups. After 8 weeks, hearts were isolated and perfused. Isolated hearts were given a 15-min stabilization period, during which end-diastolic pressure was set to 10 mmHg by adjusting balloon volume. Thereafter, pressure–volume curves were obtained as described above. Hearts were allowed to restabilize at EDP = 10 mmHg. One 5-min ‘one-pulse’ \textsuperscript{31}P NMR spectrum was then recorded. A saturation transfer measurement of CK reaction velocity followed. Finally, another 5-min ‘one-pulse’ \textsuperscript{31}P NMR spectrum was recorded to test for metabolic stability during the saturation transfer measurement. At the completion of the protocol, hearts were saved as described for determination of infarct size and measurement of creatine content. In addition, at the time of sacrifice, blood was collected and centrifuged for determination of serum creatine levels.

2.9. Statistical analysis

Results were compared using an unpaired, Bonferroni-corrected t-test [25]. The following comparisons between groups were made: Sh vs. Sh+Cr, Sh vs. MI, MI vs. MI+Cr and Sh+Cr vs. MI+Cr. With a maximum of two comparisons per group (e.g. Sham vs. MI, Sham vs. Sham+Cr), p values <0.025 were considered to indicate statistical significance.
Table 1

Characteristics of treated and untreated, sham and MI hearts

<table>
<thead>
<tr>
<th>n</th>
<th>Sh</th>
<th>MI</th>
<th>Sh+Cr</th>
<th>MI+Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI size (%)</td>
<td>7</td>
<td>0</td>
<td>36±1</td>
<td>37±2</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.84±0.06</td>
<td>2.14±0.10</td>
<td>1.98±0.07</td>
<td>2.25±0.20</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>529±31</td>
<td>555±17</td>
<td>589±11</td>
<td>559±17</td>
</tr>
<tr>
<td>HW/BW (%)</td>
<td>3.47±0.26</td>
<td>3.85±0.13</td>
<td>3.38±0.17</td>
<td>3.99±0.36</td>
</tr>
<tr>
<td>HR (l/min)</td>
<td>256±8</td>
<td>231±17</td>
<td>253±17</td>
<td>254±16</td>
</tr>
<tr>
<td>CF (ml/min)</td>
<td>30.3±1.4</td>
<td>23.3±2.1 b</td>
<td>29.0±1.0</td>
<td>28.9±1.2</td>
</tr>
<tr>
<td>CF/HW (ml/min/g)</td>
<td>17.2±1.4</td>
<td>12.6±1.1</td>
<td>14.8±1.1</td>
<td>15.6±2.0</td>
</tr>
</tbody>
</table>

a Infarct size (MI size), heart weight/body weight ratio (HW/BW), heart rate (HR), coronary flow (CF); values for coronary flow/heart weight (CF/HW) are normalized for mass of perfused tissue.

b p<0.025 Sham vs. MI.

3. Results

3.1. Infarct size, heart and body weights

Table 1 shows infarct sizes, heart weights, body weights and their ratios for the four experimental groups. Infarct size was similar for creatine-treated and untreated infarcted animals (36 vs. 37%). Inspite of the loss of almost 40% of left ventricular tissue due to infarction, marginally increased heart weights and heart weight/body weight ratios in both MI groups attest to hypertrophy of residual intact myocardial tissue due to remodeling. Creatine feeding of MI hearts did not change heart weights or heart weight/body weight ratios.

3.2. Mechanical performance and coronary flow

Fig. 1 shows pressure–volume curves for left ventricular volume and developed pressure from all experimental groups. As it is typical for this model, infarcted groups showed a displacement of the pressure–volume curve towards higher volumes (dilatation) and lower developed pressures (dysfunction). During chronic creatine feeding, pressure–volume curves did not change significantly for both sham and MI hearts. In addition, Table 1 shows that heart rate (recorded at EDP = 10 mmHg) was similar for all experimental groups. Therefore, creatine feeding did not affect cardiac function or structure in normal or in chronically failing heart. Table 1 also shows changes in coronary flow. Both in absolute (p<0.025) and relative (p<0.05) terms, coronary flow was significantly reduced in the untreated MI group. Interestingly, however, this decrease of coronary flow was prevented by chronic creatine feeding of infarcted hearts.

3.3. Energy metabolism in sham and infarcted, treated and untreated hearts

Fig. 2 shows typical 31P NMR spectra from the four experimental groups. The spectra demonstrate the reduction of the phosphocreatine resonance area that occurs post-MI. This was observed for both untreated and creatine-treated animals. Table 2 shows mean metabolite concentrations for the experimental groups. ATP concentrations were assumed to be as previously described (see Section 2); ATP levels remain constant in this heart failure model. Creatine feeding did not alter phosphocreatine content in sham hearts, and phosphocreatine levels were reduced to 81% in untreated MI and to 83% in treated MI groups; similarly, free creatine levels were reduced to 65 and 74%, respectively. Thus, chronic creatine feeding did not significantly alter the contents of phosphorylated and non-phosphorylated creatine in sham and in MI hearts. Also, cytosolic phosphorylation potentials were not significantly different among groups. Inorganic phosphate (Pi) was low in all experimental groups, the statistically significant increase of Pi in creatine-fed MI hearts was small in absolute terms (+1.4 mmol/l). Intracellular pH was similar for all groups.

Table 2 also shows results of saturation transfer measurements of CK kinetics. Compared to sham hearts, where creatine feeding had no effect, CK reaction velocity (‘energy reserve via CK’) decreased by 35% in untreated and by 53% in treated MI groups. Thus, creatine feeding did not increase CK reaction velocity in either sham and MI hearts. Therefore, our data demonstrate that chronic creatine feeding had no effect on cardiac function and steady state as well as dynamic high-energy phosphate
metabolism in both normal and failing hearts. Serum creatine levels were not significantly different between sham and MI hearts and were increased ~2-fold in both creatine-treated groups.

4. Discussion

4.1. Definition of the model

In the present study, we examine possible beneficial effects of chronic creatine feeding on cardiac function and energy metabolism in a well-established model of heart failure, i.e. in the rat heart post-myocardial infarction. Using this model, we have previously defined left ventricular dilatation, systolic and diastolic dysfunction, as they occur 2 months after coronary artery ligation in the isolated heart model [1,10,26], as well as in vivo [27]. In residual intact myocardium, ATP levels remain unchanged [1], phosphocreatine and free creatine levels decrease by up to 40% [1,16] and the creatine kinase reaction velocity is reduced by ~50% [1]. All these changes could be

Table 2

Steady-state concentrations of high- and low-energy phosphates, cytosolic phosphorylation potential, intracellular pH, creatine kinase reaction velocity and serum creatine content

<table>
<thead>
<tr>
<th></th>
<th>Sh</th>
<th>MI</th>
<th>Sh+Cr</th>
<th>MI+Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (mmol/l)</td>
<td>Set to 10.8</td>
<td>Set to 10.6</td>
<td>Set to 10.8</td>
<td>Set to 10.6</td>
</tr>
<tr>
<td>PCr (mmol/l)</td>
<td>14.2±1.7</td>
<td>11.5±0.8*</td>
<td>15.5±1.2</td>
<td>12.9±0.5*</td>
</tr>
<tr>
<td>Free Cr (mmol/l)</td>
<td>15.5±0.7</td>
<td>10.0±0.8*</td>
<td>15.1±0.8</td>
<td>11.1±1.0*</td>
</tr>
<tr>
<td>P (mmol/l)</td>
<td>2.5±0.4</td>
<td>2.7±0.2</td>
<td>2.8±0.4</td>
<td>4.2±0.3*</td>
</tr>
<tr>
<td>pH</td>
<td>7.15±0.01</td>
<td>7.17±0.01</td>
<td>7.16±0.00</td>
<td>7.14±0.02</td>
</tr>
<tr>
<td>CK flux (mmol/l/s)</td>
<td>12.0±0.7</td>
<td>7.8±0.7*</td>
<td>13.6±1.0</td>
<td>7.2±1.1*</td>
</tr>
<tr>
<td>PP (10^3 M^-1)</td>
<td>37.5±5.3</td>
<td>39.4±1.2</td>
<td>36.6±4.0</td>
<td>31.5±2.2</td>
</tr>
<tr>
<td>Cr_serum (mmol/l)</td>
<td>0.54±0.10</td>
<td>0.43±0.08</td>
<td>1.00±0.11*</td>
<td>0.88±0.10*</td>
</tr>
</tbody>
</table>

* Phosphocreatine (PCr), creatine (Cr), inorganic phosphate (P), creatine kinase reaction velocity (CK flux), cytosolic phosphorylation potential (PP).

ATP concentrations were assumed to be as previously determined by HPLC (10.8 mmol/l in sham and 10.6 mmol/l in MI; see Section 2).

* \( p<0.025 \) Sham vs. MI.

* \( p<0.025 \) treated vs. untreated.
reproduced for the MI group in the present study. We also previously showed that pharmacologic interventions such as angiotensin-converting-enzyme inhibitors [28] or beta-receptor blockers [10] were able to fully or partially reverse the functional and energetic deterioration occurring after MI. Thus, we studied a heart failure model where functional and energetic derangements are well defined and where beneficial pharmacologic interventions can be demonstrated to afford protection.

Our approach to increase myocardial creatine and, thus, as it was hoped, phosphocreatine levels, was to chronically increase the extracellular creatine supply to the heart by feeding high dosages of creatine with the diet for 2 months. In the present work, 3% creatine feeding increased serum creatine levels significantly ~2-fold in both sham and MI groups. At the same time, body weights were unaltered by creatine feeding. Thus, we tested the effect of chronically doubling the extracellular creatine supply to the heart on the development of mechanical and energetic derangement following coronary ligation.

4.2. Effects of oral creatine supplementation on mechanical function and energy metabolism in heart failure

Whether changes in high-energy phosphate metabolism contribute to contractile dysfunction in heart failure is an old and still unresolved question [9]. Although direct proof is missing, it is conceivable that reduction of energy reserve via creatine kinase and failure to maintain a high cytosolic phosphorylation potential and free energy change of ATP hydrolysis (ΔG) [24] are mechanisms contributing to cardiac failure. In the present work, we attempted to prevent the decrease of phosphocreatine and free creatine in heart failure by increasing creatine supply to the heart. In case this intervention were successful, we could test whether maintenance of high (phospho-) creatine content has beneficial functional effects.

In the mammalian organism, creatine is synthesized by liver and kidney and, in addition, is supplied with a non-vegetarian diet (see Ref. [29] for a review). Cardiomyocytes lack the enzymes required for creatine synthesis and, instead, accumulate creatine via a specific membrane protein, the Na⁺-creatine cotransporter, which has recently been cloned [12], against a large concentration gradient [12,29]. Intracellularly, creatine is phosphorylated to phosphocreatine via creatine kinase. The degradation of phosphocreatine and creatine occurs non-enzymatically via spontaneous decarboxylation and passive transmembrane diffusion [12], processes unlikely to be regulated. Therefore, the obvious approach to attempt to increase intracellular creatine content is to increase the substrate supply to the creatine transporter by raising extracellular creatine levels. Previous work has shown that in human skeletal muscle, this approach is successful: Here, total creatine content can be increased by 17% with dietary creatine supplementation [13]. Species differences may exist, however, since in rat, skeletal muscle creatine content could not be increased by creatine feeding [15]. In skeletal muscle, a 17% increase of creatine levels has functional relevance, and maximum work output increases significantly (see Ref. [14] for a review). Based on these observations, it was conceivable that the same approach would also be effective for cardiac muscle. Although it was shown before that normal cardiac muscle does not increase its creatine content with increased serum creatine levels [15], it was possible that this intervention is effective for conditions where creatine is chronically depleted. Furthermore, the regulation of myocardial creatine uptake and degradation in heart failure is still completely unknown. We know of no published clinical study of the effects of creatine treatment in heart failure patients, but there are some initial studies related to beneficial effects of phosphocreatine treatment (e.g. [30,31]), where acute and subacute improvement of cardiac function was observed. However, the creatine transporter does not transport phosphocreatine, and no other mechanism for direct uptake of the polar compound phosphocreatine by cardiomyocytes has been demonstrated. Thus, if phosphocreatine does have a beneficial effect in heart failure, it is not related to improvement of cardiac energetics but, likely, rather due to some indirect (vascular, electrophysiologic or other) effect.

The results presented here unequivocally demonstrate that the strategy to chronically provide high dosages of creatine to the failing heart is ineffective in preventing the decrease of creatine content and of energy reserve via creatine kinase. Inspite of chronically doubling extracellular creatine concentrations, myocardial phosphorylated and non-phosphorylated creatine levels as well as creatine kinase reaction velocity all remained unaffected. In light of these findings, it was not surprising that creatine feeding had no effect on functional consequences of left ventricular remodeling post-MI with the exception of maintenance of coronary flow. Since heart creatine levels could not be increased with higher serum creatine levels, we could not test the hypothesis that maintaining high cardiac creatine levels during the development of heart failure has beneficial functional effects. Therefore, inspite of a lack of effect of creatine feeding, our results do not argue against a causal role of energy metabolism in heart failure, but also do not provide new evidence in support of this concept.

We can only speculate on the reasons why higher extracellular creatine levels are ineffective in increasing creatine levels and mechanical work in the normal or failing heart. There are currently no data available on the regulation of creatine transport under in vivo conditions, and speculations have to rely on results obtained from cell culture models, none of which have used cardiomyocytes from failing hearts. Creatine transporter kinetics were shown to be dependent on Na/K-ATPase activity, β₂-receptor stimulation and thyroid hormone status [32], but
none of these mechanisms is likely to bear on our studies. One possible explanation, however, is that both in human muscle cells as well as in G8 myoblasts, creatine transporter activity was inversely related to extracellular creatine supply, i.e. was downregulated by high and upregulated by low extracellular creatine concentrations [33]. Thus, the most likely explanation for the failure of dietary creatine to increase heart creatine stores is that the creatine transporter activity is downregulated under these circumstances, thereby preventing an increase of intracellular creatine concentrations. In our study, creatine transporter levels were not directly determined, and it will be important to do this in future studies in order to understand the regulation of this transporter in normal and diseased heart. A second explanation for the lack of effect of creatine feeding could be that in cell culture, creatine uptake is saturated at ~500 μM and can, thus, be increased no further by increasing extracellular creatine concentrations from ~500 to ~1000 μM, corresponding to serum levels of untreated and treated groups in our study. It is unknown, however, whether saturating concentrations are different for normal and failing heart. Whatever the reasons for a lack of effect, our study demonstrates that providing high extracellular creatine concentrations is not an effective treatment approach in heart failure in terms of improving myocardial function. Thus, other strategies to increase creatine content in heart failure will have to be developed. Specific stimulators of creatine transport are currently unknown. One approach that may be effective is to increase creatine transporter protein content, either via gene transfer or transgenic overexpression models. These studies remain to be done.

An unexpected finding was that creatine feeding prevented the small but significant decrease of coronary flow occurring post-MI, while coronary flow in sham hearts was unaffected by creatine. It is unlikely that this can be explained by an acute vasodilator effect of creatine, since hearts were perfused under creatine-free conditions, and since in sham operated hearts, creatine did not alter coronary flow. In addition, perfusion of isolated rat hearts with 1 mmol/l creatine did not acutely affect coronary flow (n = 3, data not shown). It is, thus, conceivable that chronic creatine feeding has effects on the coronary microcirculation. Data to this point are currently lacking, and this observation may warrant further study. Whatever the reasons, however, maintenance of higher global coronary flow levels by creatine feeding in MI hearts did not have any beneficial functional or energetic consequences. Furthermore, inorganic phosphate levels were slightly, yet significantly, higher in creatine-treated MI hearts. The reason for this is unclear, but the small absolute magnitude of the change (+1.4 mmol/l) and the fact that the amount of total NMR observable phosphates was the same for all groups (data not shown) suggest that this finding does not have physiological significance. In addition, cytosolic phosphorylation potential did not change significantly, again arguing against a pathophysiological role for the increase of inorganic phosphate in creatine-treated MI hearts.

In summary, we have shown that chronically providing failing rat hearts with increased extracellular creatine fails to elicit beneficial structural, functional and energetic effects. The additional creatine is not accumulated by the heart. Thus, creatine treatment does not seem to be a promising approach for the treatment of heart failure.

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References


