Increased Myocardial Energy Reservation by Two-Week Creatine Supplementation in Exercise-Trained Female Hamsters

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Abstract

Creatine phosphate (Cr) and its phophorylated form (phosphocreatine, PCr) is catalyzed by creatine kinase (CK) reaction to phosphorylate ADP to ATP, which plays an important role in muscle energetics. This study aims to examine the combined effect of Cr and/or exercise training on myocardial energy reservation, using female hamsters. Fifty female Golden Syrian hamsters were divided into 4 groups of control (n=10), Cr supplementation (Cr+; n=10), exercise training (Ex; n=10), and Cr supplementation combined with exercise training (Cr+Ex; n=10). In the exercise-trained group, wheel-running for exercise (10 min. a day, 5 days a week) was imposed for 2 weeks. All animals were measured for exercise metabolic rate (EMR) on the 1st, 7th and 14th day. After the animals were sacrificed, the hearts were cut and weighed and the contents of Cr, PCr, total Cr (TCr), CK activities and RNA content were measured, using a spectrophotometric method.

The animals treated with Cr+Ex on the 2nd week yielded the greatest EMR level with the lowest of oxygen consumption, compared with Cr+ or ex alone. Additionally, all parameters regulating myocardial energy reservation (contents of Cr, PCr, TCr and CK activity) and RNA contents improved greatly in Cr+Ex, treatment. These data indicate that creatine supplementation, when combined with exercise training, have many benefits for oxygen economy and effectiveness for myocardial energy reservation via Cr metabolism.

Key words: Creatine, energy reservation, exercise, hamsters, myocardium.

1. Introduction

Creatine (Cr) enhances muscle performance, especially in skeletal muscle [1]. At the onset of muscle work, Cr alters the energy cost of muscle force production (ATP cost) by increasing PCr content in skeletal muscle [2]. However, less is known about the beneficial effect of Cr on cardiac muscle. Depletion of creatine (Cr) in the cardiac muscle leads to a reduction in cardiac performance [3]. Recent results show that a loss of myocardial antioxidant reserve under Cr depletion had an effect on

electrical and mechanical damage in response to an increase in oxidative stress [4]. Other studies in failing hearts of humans and animals demonstrated a reduction in the creatine kinase (CK) system [5] and its products, Cr and phosphocreatine (PCr) contents [6].

Supplementation of Cr can improve myocardial energy and performance in patients with heart diseases [7, 8]. This suggests that an increase in intracellular Cr levels may increase the myocardial energy reserve via the creatine kinase (CK) system [9] and a specific Cr transporter (CrT) [10]. Additionally, regular physical exercise may elicit positive adaptation, which results in an improvement of myocardial functions [11, 12].

Cr-supplementation, and exercise training may have important roles in cardiac muscle. It is as yet unclear whether the Cr-supplementation and/or exercise training, have benefits for oxygen economy and effectiveness for myocardial energy reservation. In this study, we examined those combined effects using female hamsters.

2. Materials and methods

2.1 Animals and experimental procedures

Fifty female Golden Syrian hamsters (body weight (BW): 100-120 g; National Laboratory Animal Centre of Mahidol University, Salaya, Thailand) were used for this study. The hamsters were fed standard chow and water *ad libitum*. The animal experiment was conducted according to the guideline on experimental animals of The National Research Council of Thailand (1999).

The animals were divided into 4 groups of control (C), Cr supplementation (Cr+, Cr monohydrate 200 mg/kg BW, orally), exercise training (Ex, wheel-running exercise), and Cr supplementation combined with exercise training (Cr+Ex, Cr monohydrate 200 mg/kg BW plus wheel-running exercise). All treatments were performed daily, and continued for 2 weeks.

In the exercise-trained group, wheel-running exercise (40 rounds per minute) was imposed for 2 weeks (10 minutes a day, 5 day a week). All animals were measured at resting metabolic rate (RMR) on the day before experiment and at exercise metabolic rate (EMR) on the 1st, 7th and 14th day of experiment, using a closed circuit calorimeter (a metabolic chamber) at an ambient temperature (23$\pm$2 ^{o}C), and expressed as cal/m^2/hr [13]. Oxygen economy was calculated as the oxygen consumption from EMR on day 14 and normalized for distance traveled, expressed in ml O_2/kg/m [14].

2.2 Biochemical assay

2.2.1 Myocardial energy

After the animal was sacrificed, the heart was cut and weighed. It was rapidly frozen in liquid nitrogen, and stored at -70 ^{o}C until further analysis of myocardial energy reservation. Metabolic phosphate contents were measured by adapting the nonenzymatic technique [15]. The cardiac muscle was initiated in a homogenization buffer (0.5 M $HClO_4$ in 1 mM EDTA) and centrifuged at 10,000 rpm at 4 ^{o}C for 2 minutes. After the supernatant was re-centrifuged at 10,000 rpm at 4 ^{o}C for 2 minutes, the reaction tube (0.25 ml of sample; 0.5 ml of diluted diacetyl, 1 ml of 1 % α-napthol) was placed in the dark for 40 minutes, and measured using a spectrophotometer at 520 nm. The PCr concentration was calculated from the difference between TCr and Cr for each sample and expressed as μmole/mg protein.

CK activity was measured using a modified protocol as follows [16]. Tissue was placed in 10 volumes of a cold isotonic extraction buffer (250 mM sucrose, 50 mM imidzole acetate, 10 mM Mg acetate, 4 mM KH_2PO_4, 2mM EDTA, 50 μM N-acetylcysteine and 12.5 μM sulfur in 0.8 % ETOH, pH 7.6). After centrifugation at 2,000 x g at 4 ^{o}C for 5 min., the supernatant

was centrifuged for 10 min. The final supernatant was analyzed with CK-NAC UV liquid (Biochemica and Diagnotica mbH, Germany) on the spectrophotometer at 340 nm at 25 ^{o}C and expressed as μmol/min/mg tissue protein.

2.2.2 Total RNA contents

Total RNA content was quantified as the potential for an altered capacity for cardiac muscle protein synthesis following the method of Fleck and Munro [17]. Cardiac tissue was weight and homogenized in 0.2 N perchoric acid ($HCLO_4$) using a Homogenizer (Glas-COL, Indiana 47802) at 60% of maximum setting (4x10 sec each) on ice. After several washes the pellet was resuspended in 0.3 N KOH. Then 0.75 ml of 0.2 N $HClO_4$ was added to the supernatant. After centrifugation (12,000x g at 4 ^{o}C for 10 minutes), the supernatant was transferred to a new tube. The pellet was washed two more times with 0.2 N $HClO_4$ and the supernatants from all washes were combined for total RNA quantified by UV absorbance at 260 nm (Shumudzu UV1201, Japan). Total RNA, is expressed as the concentration per mg of cardiac muscle [18].

2.3 Statistical analysis

All data are expressed as the mean and standard deviation (mean±SD). Results were analyzed with one-way repeated measure design (on the 1st, 7th and 14th day within the same group) analysis of variance (ANOVA). For other results, data were analyzed using one way ANOVA. If significance was obtained with ANOVA, a post hoc method (Student-Newman-Keuls test) was used to test the differences between groups. The level of significance for all analysis was set at $p<0.05$.

3. Results

3.1 Body weight and heart weight

As shown in Table 1, BW, HW and HW/BW ratio were significantly increased from 86.9 ± 2.60 g, 0.34 ± 0.02 g and 4 ± 0.1(x10^{-3}), respectively, in the control, to 101.5 ± 1.6, 0.46 ± 0.02 g and 4.61± 0.08 (x10^{-3}), respectively, in female hamsters treated with Cr+Ex ($p<0.05$).

3.2 Metabolic rate and oxygen economy

There were no differences in metabolic rate at rest among control, Cr+, Ex and Cr+Ex groups (data not shown). EMR on the 7th day was significantly increased in Ex and Cr+Ex hamsters ($p<0.05$) when compared to the control. Interestingly, EMR in female hamsters treated with Cr+Ex was profoundly enhanced on the 14th day (con= 62 ± 0.2, Cr= 64.1 ± 2.2, Ex= 67.3 ± 1.4, Cr+Ex= 74.1 ± 1.6 cal/m^2/h; $p<0.05$, Fig.1).

Oxygen economy values of 4 groups of animals were calculated from oxygen consumption of EMR on the 14th day of experiment. The results showed that female hamsters treated with Cr+Ex for 14 days had decreased oxygen economy as compared to the others (con= 3.63 ± .09, Cr= 3.41 ± 0.09, Ex= 3.35 ± 0.07, Cr+Ex= 3.23 ± 0.04 ml O_2/kg/m; $p<0.05$, Table 1).

3.3 Myocardial metabolic phosphate contents

As shown in Table 2, metabolic phosphate content of Cr, PCr and TCr on the 14th day in the control were 3.7 ± 1.0, 5.9 ± 2, 9.0 ± 1.0 x10^{-2}μmol/mg protein, respectively. A marked increase in myocardial Cr content was observed in Cr+, Ex and Cr+Ex animals, which were 8.0 ± 2.0, 10.0 ± 1.0, and 18.6 ± 2 x10^{-2} μmol/mg protein, respectively ($p<0.05$). Similar to Cr content, the content of TCr was also increased in all treated groups. In contrast, an accumulation of PCr content was observed only in Cr+Ex animals, which were 13.0 ± 2.0 x10^{-2} μmol/mg protein ($p<0.05$). Interestingly, the contents of all myocardial metabolic phosphate contents (Cr, PCr and TCr) were the highest in the animals treated with Cr+Ex, compared to the others ($p<0.05$).

3.4 Myocardial CK activities and RNA contents

Animals treated with Cr+ on the 14th day significantly increased the activity of CK is compared with the control value of 7.5 ± 0.9 to 9.9 ± 0.7 μmol//mg protein ($p<0.05$, Table 2). A greater increase in CK activities could be seen in Ex and Cr+Ex animals (14.3 ± 0.9 and 15.2 ± 1.2 μmol/mg protein, respectively; $p<0.05$). After 14 days of experiment, RNA contents were increased in all treated groups of Cr+, Ex and Cr+Ex, which were 2.5 ± 0.2, 3.4 ± 0.1 and 3.6 ± 0.6 μg/mg cardiac muscle, respectively ($p<0.05$, Table 2). Similar to myocardial CK activities, the RNA contents were improved greatly in Ex and Cr+Ex female hamsters.

Table 1. Effect of Cr supplementation and exercise training on body weight (BW), heart weight (HW), heart weight/body weight (HW/BW) ratio and oxygen economy (OE) on the 14th day in female hamsters.

Group	BW (g)	HW (g)	HW/BW ratio (x 10^{-3})	OE (ml O_2/kg/m)
Con (n=10)	86.9±2.6	0.34±0.02	4.0±0.1	3.63±0.09
Cr (n=10)	89.5±0.6	0.35±0.04	3.9±0.4	3.41±0.09
Ex (n=10)	88.0±1.60	0.38±0.02	4.3±0.08	3.35±0.07
Cr+Ex (n=10)	101.5±1.6*	0.46±0.02*	4.6±0.08*	3.23±0.04*

The data are presented as mean±SD. Control (C), Cr supplementation (Cr+), exercise training (Ex) and Cr supplementation combined with exercise training (Cr+Ex). *P<0.05 are significantly different *vs* any groups (C, Cr+, Ex, Cr+Ex).

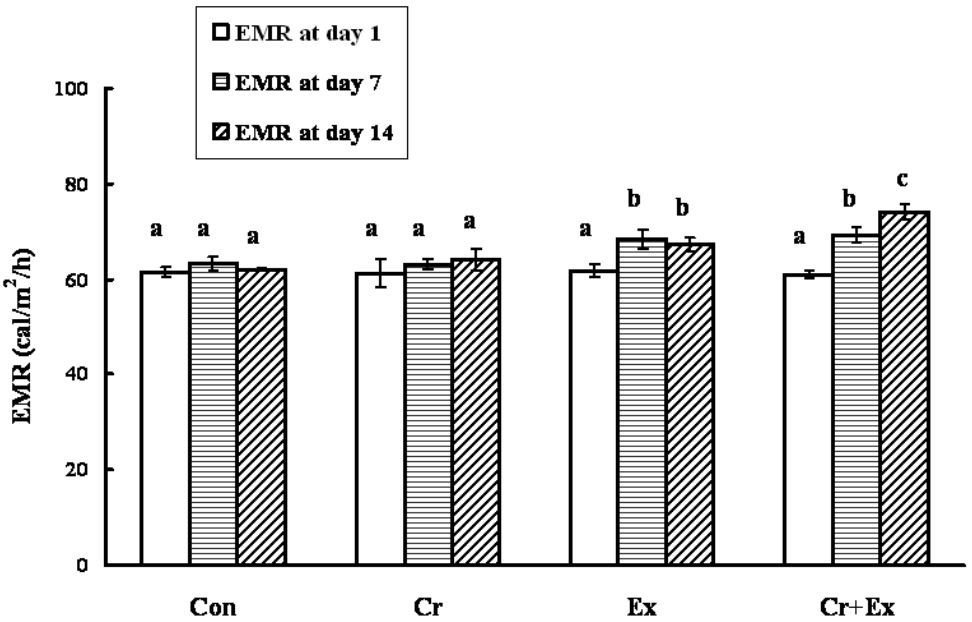

Fig.1 Effect of Cr supplementation and exercise training on EMR on the 1st, 7th and 14th day. The data are presented as mean±SD. Control (C), Cr supplementation (Cr+), exercise training (Ex) and Cr supplementation combined with exercise training (Cr+Ex). Two groups of

different superscripts (a-c) are significantly different from one another among periods in the same treatment, for female hamsters ($P<0.05$).

Table 2. Effect of Cr supplementation and exercise training on metabolic phosphate contents (Cr, PCr and TCr), CK activities and RNA contents on the 14th day in female hamsters.

Group	Cr	PCr	TCr	CK	RNA
	($x10^{-2}$ μmol/mgprotein)			(μmol/mgprotein)	(μg/mg muscle)
Con (n=10)	3.7+1.0[a]	5.9+2.0[a]	9.6+3.0[a]	7.5+0.9[a]	2.1+0.1[a]
Cr (n=10)	8.0+2.0[b]	7.2+2.0[a]	15.2+4.0[b]	9.9+0.7[b]	2.5+0.2[b]
Ex (n=10)	10.1+1.0[b]	7.7+1.0[a]	17.8+2.0[b]	14.3+0.9[c]	3.4+0.1[c]
Cr+Ex (n=10)	18.6+2.0[c]	13.0+2.0[b]	31.6+4.0[c]	15.2+1.2[c]	3.6+0.6[c]

The data are presented as mean±SD. Control (C), Cr supplementation (Cr+), exercise training (Ex) and Cr supplementation combined with exercise training (Cr+Ex). In each data (Cr, PCr, TCr, CK and RNA), two groups with different superscripts (a-c) are significantly different from one another ($P<0.05$).

4. Discussion and conclusion

In our study, BW, HW and HW/BW ratios in hamsters treated with Cr+ were not different from those of the control (Table 1). It is implied that animals appeared to tolerate the Cr feeding regimens, with no signs of distress [19]. Additionally, short-term exercise training (Ex, 10 minutes a day, 5 days a week for 2 weeks) did not show BW loss or cardiac hypertrophy. In contrast, BW and HW exhibited higher sensitivity in hamsters treated with Cr+Ex than the others. This change can be explained by Volek et al. [20] who suggested that exercise training can increase muscle fibers in both type I and type II in human with Cr supplementation.

Our results showed no relationship between the Cr feeding and the EMR level, It may be because Cr has no effect on O_2-uptake. In other groups of hamster treated with Ex and Cr+Ex, the level of EMR on the 7th day increased significantly compared to the control. This indicates wheel-running exercise in our study was enough to increase O_2-uptake, providing for cardiac consuming capacity. In addition, EMR in Cr+Ex hamsters were progressively increased with increasing time of experiment on the 14th day, compared with Ex treatment alone. The increase may be because Cr was more affected by EMR level when combined with exercise training. As an effect of exercise training, Casay et al. [21] suggested a restoration in ATP–producing capacity system via Cr metabolism. Cr feeding combined with exercise training may increase O_2-uptake, which is reinforced by increasing the ATP–producing capacity in cardiac muscle.

Economy has a specific sports science definition, which is defined as the energy expenditure used at a given workload. In the present study, oxygen economy is calculated from the oxygen uptake of sub-maximal running, normalized for distance traveled on the 14th day of the experiment. Our results showed a significant reduction of oxygen economy during exercise in all treated groups, compared to the control. The extent of reduction of oxygen economy was the highest in Cr+Ex hamsters. As to the effect of Cr in exercise animals: it caused more oxygen to be saved, which might be from the reduction of energy expenditure, at the same given workload when compared to the others.

In the present experiment, Cr and TCr contents were significantly increased in Cr-fed on the14th day, which agrees with a previous study [22]. Additionally, hamsters with Ex on the 14th day also had a marked increase in Cr and TCr contents. It is well conceivable that changes in Cr metabolism contribute to cardiac contractile dysfunction [4, 23]. This data showed that exercise training improves cardiac function, by increasing myocardial energy reserve via Cr metabolism. Interestingly, Cr+Ex hamsters had enhancement of all metabolic phosphate contents (Cr, PCr and TCr), when compared with Cr+ or Ex treatment alone. Harris et al [24] suggested that exercise training can influence the net uptake of Cr into muscle. Accordingly, Cr treatment combined with exercise training, was more effective for Cr-uptake and activity and the CK energy shuttle was increased because of enhancement of blood flow [25].

CK activity was increased significantly after Cr+ treatment on the 14th day, compared to the control. This indicates that Cr+ treatment should play an important role in activation of metabolic phosphate contents and CK activity in cardiac muscle. Interestingly, CK activity was improved greatly in hamsters by treatment of Ex and Cr+Ex.

As to the content of RNA: it indicates that Cr+ treatment on the 14th day in our study was enough to change the stimulus for promoting the protein synthesis. According to Merrick [26], endurance exercise showed an ability to alter RNA abundance within cardiac muscle, leading to an increase in protein synthesis and hypertrophy. It was apparent in our study that hamsters treated with Ex alone can increase protein synthesis, but did not exhibit the cardiac hypertrophy. Consequently, Cr+Ex hamsters may improve in cardiac protein synthesis, and have the mechanical strain necessary for cardiac hypertrophy.

Currently, the mechanism of Cr in inducing energy reservation has become an interesting subject, particularly in athletes. Cr is absorbed into the bloodstream, most likely by the amino acid transporter, CrT, and is transported to the muscle cell [27]. Cr uptake can be massively upregulated in the heart by increasing the total CrT mRNA levels [10]. The ergogenic effects of Cr have been reported. Cr might directly stimulate ATP synthesis in muscle and hence, PCr causes reaction with ADP to form an ATP from an ATP-producing capacity system, resulting in a prolongation of muscle working [28]. Supplementation with Cr has been shown to increase physical working capacity by delaying neuromuscular fatigue [29]. In addition, recent studies have confirmed that Cr supplementation can prevent protein degradation [30], increase mitotic activity of satellite cell proliferation, myogenic transcription factors and insulin-like growth factor-1 signalling [31].

In conclusion, it is clear from the present study that, Cr-feeding or exercise training for 14 days improved myocardial energy reservation via Cr metabolism. All parameters regulating myocardial Cr metabolism, and oxygen economy improved greatly in Cr-feeding combined with exercise training in hamsters. Up to this point, it is worth suggesting a combined beneficial effect of Cr-feeding in exercise hamsters on oxygen economy, as well as myocardial energy reservation, via Cr metabolism. However, the mechanism of these combined effects related to CrT protein or cardiac pumps should be further studied.

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6. References

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