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Research article

Exercise performance and muscle contractile properties after creatine monohydrate supplementation in aerobic-anaerobic training rats

Nickolay Boyadjiev [1]✉, Dobrin Popov [1] and Slavi Delchev [2]
[1]Department of Physiology, Medical University of Plovdiv, Bulgaria, [2] Department of Anatomy, Histology and Embryology, Medical University of Plovdiv, Bulgaria

Abstract
The purpose of this study was to investigate the effects of creatine monohydrate supplementation on exercise performance and contractile variables in aerobic-anaerobic training rats. Twenty 90-day-old male Sprague Dawley rats were divided into two groups - creatine (Cr) and controls (K). The creatine group received creatine monohydrate as a nutritional supplement, whereas the control group was given placebo. Both groups were trained 5 days a week on a treadmill for 20 days in a mixed (aerobic-anaerobic) metabolic working regimen (27 m·min^{-1}, 15% elevation for 40 min). The exercise performance (sprint-test), contractile properties (m. tibialis anterior), oxidative enzyme activity (SDH, LDH, $NADH_2$) in m. soleus and blood hematological and chemical variables were assessed in the groups at the end of the experiment. It was found out that creatine supplementation improved the exercise performance after 20 days of administration in a dose of 60 mg per day on the background of a mixed (aerobic-anaerobic) exercise training. At the end of the trial the Cr-group demonstrated better values for the variables which characterize the contractile properties of m. tibialis anterior containing predominantly types IIA and IIB muscle fibers. On the other hand, a higher oxidative capacity was found out in m. soleus (type I muscle fibers) as a result of 20-day creatine supplementation. No side effects of creatine monohydrate supplementation were assessed by the hematological and blood biochemical indices measured in this study.

Key words: Exercise, creatine supplementation, rats, performance, muscle contractile properties.

Introduction

Recently, there has been a considerable scientific interest (Kamber et al., 1999) shown in creatine as an ergogenic aid for improving exercise performance. Creatine supplementation first gained popular attention in the early 1990s after high profile Olympic athletes competing in sprint and power events at the Barcelona Olympic Games believed that it was creatine that had a beneficial impact on their performance. Since this time creatine has become one of the most widely used nutritional supplements with an estimated worldwide consumption of 2.7 million kilograms (Williams et al., 1999). Creatine is not included in the International Olympic Committee list of banned substances. Most of the consumers of this nutritional supplement are power athletes, sportsmen with predominantly anaerobic working regimen of muscles. Harris et al. (1992) found that phosphocreatine (PCr) content in muscles can increase up to 50% following daily creatine supplementation. As a result, an increase in total creatine stores may provide an ergogenic effect during high intensity exercise by enhancing the rate of ATP synthesis during contraction and by improving the rate of PCr resynthesis during recovery, which may be beneficial for repeated power or sprint activity, for example. Most of the studies investigating the effect of creatine supplementation are performed on subjects (humans or experimental rats) that do strength/power/sprint sports and do work with maximal effort of muscle contractions (Mujika et al., 2000; Vandenberghe et al., 1997; Volek et al., 1999). There have been few studies, however, performed on aerobic-anaerobic training subjects.

The aim of this study was to examine the effects of creatine monohydrate supplementation of the diet on exercise performance and contractile variables in a mixed aerobic-anaerobic training regimen in rats.

Methods

We used 20 Sprague-Dawley rats approximately 90 days old at the baseline. The rats were housed in individual metabolic cages and were allocated into a creatine (Cr) group and a control (K) group. The experimental animals were fed the standard rat chaw ad libitum. The Cr-group received 60 mg creatine monohydrate (DSM Fine Chemicals, Austria) each day as a supplement to the standard diet (an equivalent of the recommended dose of 20 g for humans) (Ipsiroglu et al., 2001). The K-group received placebo (60 mg dextrose). Both, creatine and placebo were incorporated in minced meat balls, which were completely consumed by rats in the morning. Both groups were trained incrementally by using mixed aerobic-anaerobic workloads – five days a week on a treadmill (Columbus Instruments, Columbus, OH). The duration of the run was progressively increased every day by five minutes, starting from 25 min (27 m·min^{-1} speed and 15% slope) until the animals were running for 40 min per day (day 4). This duration was maintained till the end of the experiment (Lambert, 1990). The overall duration of the study was 20 days.

At the end of the experiment a sprint-test was performed to assess the sprinting performance of the rats. The rats ran on a treadmill (15% elevation) at 27 m/min for 3 min. The speed was then increased to 45 m/min for 30 seconds and again by 10 m/min every 30 seconds until the rat was unable to maintain the pace of the treadmill belt. The highest speed which the rat could maintain for 15 seconds was defined as the maximal sprinting speed (Lambert, 1990).

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Table 1. Body mass (g) of the experimental rats during the trial. Data are means (±SEM) .

group	starting body weight	body weight at the end of wk 1	body weight at the end of wk 2	body weight at the end of wk 3
Creatin group (n=10) *	132.8 (3.2)	158.3 (14.1)	177.3 (15.1)	187.0 (19.5)
Control (n=10)	127.8 (7.8)	153.3 (9.3)	177.4 (10.2)	194.7 (11.7)

* No significant differences compared with control group.

The contractile characteristic of m. tibialis anterior (containing predominantly types IIA and IIB muscle fibers) (Delp and Duan, 1996) of each rat in both groups was evaluated by isolating the right limb muscle under narcosis (thiopental 10 mg/kg). Each muscle was used immediately after removal. The muscle was placed in an organ bath containing Krebs-Henseleit solution, heated to body temperature and thermostatically controlled. The solution was bubbled in a carbonic mixture to maintain muscle feasibility (95% O_2 and 5% CO_2). The muscle was fixed in the organ bath by traction straps in the inferior rod and in the tensiometer in its superior part. The basic tension was adjusted to 50 gram force (gf) to each investigated muscle (1gf = 0.009807 N). The muscle was stimulated directly by using platinum electrodes placed along the long axis of the muscle. Electrical stimulation was supplied by Grass S44-type stimulator (monophasic pulses 0.5 ms in duration, 10 Hz). The appropriate stimulating voltage was determined by increasing the voltage until the measured force of contraction reached its peak measurements of the isometric-twitch curves. The force displacement transducer was connected to a special converting device which in turn was connected to the PC. The twitch curves were made using computer based software WaveRunner 1.0 (Sigma Plus, Plovdiv, Bulgaria). The following variables of the isometric contractile properties, as defined by Close and Hoh (Close and Hoh, 1968), were measured: 1. initial twitch tension (F_0), 2. maximum twitch tension (F_{max}), 3. contraction time of maximum twitch tension t_{Fmax}, 4. contraction times to F_{max}–10%, F_{max}–25%, F_{max}–50%. 5. the changes in the strength of muscle contraction (as percentage of F_{max}) during the whole time of contraction (till the 450th second from the beginning of stimulation) were also evaluated.

The right m. soleus (containing predominantly type I muscle fibers) (Delp and Duan, 1996) of each rat was prepared for histochemical analysis of the following oxidative enzymes: LDH (Hess et al., 1958), SDH (Nachlas et al., 1957), and $NADH_2$-cytochrome-c-reductase (Hess et al., 1958). The analysis of the enzyme activity was performed using Microphot microscope (Nikon, Japan) and special software DP Soft (Olympus, Japan). The intensity of enzyme activity was measured by analysis of 22080 fixed pixels (1840x12). The mean value for each field was taken for further calculations.

Blood samples were taken from each rat at the end of the experiment to determine the blood glucose, urea, creatinine, AST, ALT, LDH, CPK, and WBC, RBC and PLT count, hemoglobin, hematocrit, mean cell volume (MCV), mean concentration of hemoglobin using a hematological analyzer (CONE, Finland).

Statistical analysis: the data were analyzed with the one-way ANOVA and the significance of difference between the variation series was calculated (Statistica 6.0, StatSoft Corp.). To determine statistical significance between groups the orthogonal contrast matrix method was used (Hicks, 1973). All analyses were tested at $p < 0.05$. The data are presented as mean ± SEM.

Results

The body weight of the Cr-group rats was not significantly different from that of the control rats throughout the experiment (Table 1). This fact was important for comparison of the contractile properties of the skeletal muscles.

It is well known that the maximal sprinting test is a good predictor of the anaerobic-aerobic working capacity of the rats. At day 21 after starting the creatine supplementation and the training programme, the rats of the Cr-group demonstrated better results in their sprinting performance than the rats of the K-group achieving higher velocity of the treadmill belt during the test (79.00 ± 4.00 $m \cdot min^{-1}$ vs. 67.00 ± 3.36 $m \cdot min^{-1}$, $p < 0.05$) (Figure 1).

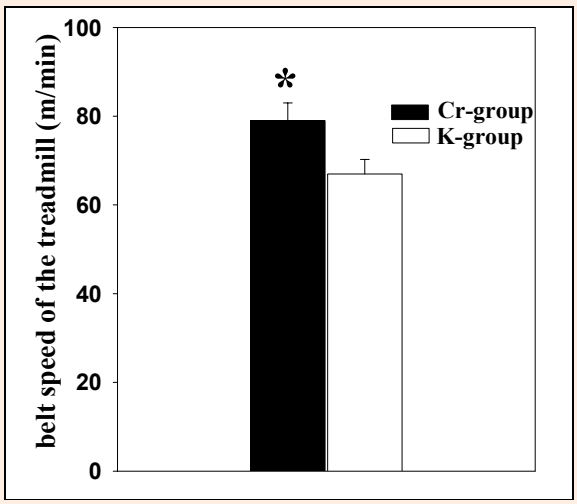

Figure 1. Sprinting test results of the groups at the end of the experiment. * $p < 0.05$.

Contractile measurements

To assess the contractile characteristics of muscles that are due to the training and creatine supplementation, m. tibialis anterior was chosen because this muscles contains predominantly fast twitch (types IIA and IIB) muscle fibers (Delp and Duan, 1996). These fibers are mainly ATP and PCr dependent for energy support. The initial twitch tension (F_0) evoked by electrical stimulation was higher in the Cr-group than that in the controls at the end of the experiment (49.74 ± 0.83 gf vs. 47.24 ± 0.81 gf $p < 0.05$). On the other hand, the maximum tension of the isolated m. tibialis anterior during continuous stimulation (F_{max}) in the Cr-group occurred later than that in the K-group (55.07 ± 1.03 s vs. 45.54 ± 0.93 s, $p < 0.001$), and

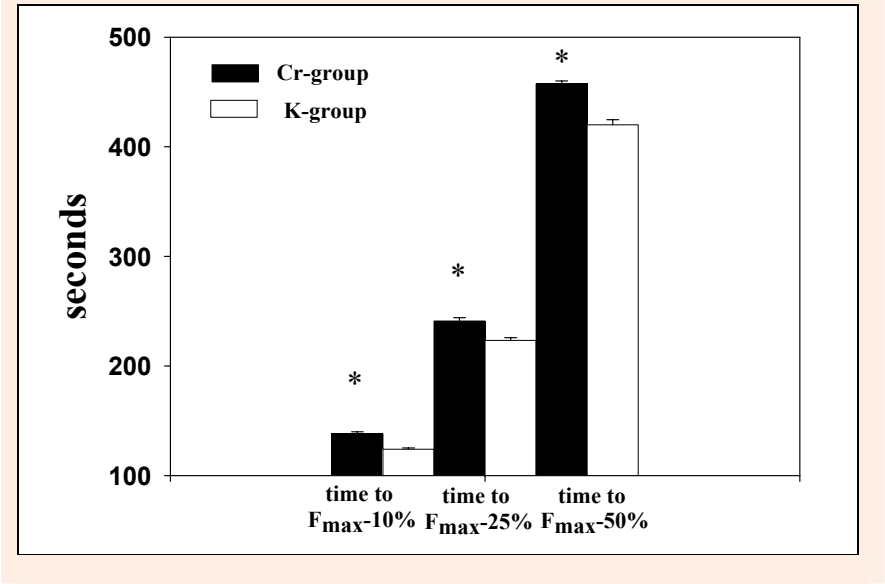

Figure 2. Contraction time to Fmax-10%, Fmax-25%, and Fmax-50%. * $p < 0.001$.

was greater that that in the controls (68.41 ± 1.19 gf vs. 63.13 ± 1.03 gf, $p < 0.01$).

The analysis of the m. tibialis anterior contraction curve following the continuous stimulation showed that the Cr-group rats delayed the time to fatigue of this muscle when compared with the controls – it took the Cr-rats longer time to reduce their F_{max} by 10%, 25%, and 50% in comparison with the K-group (138.02±1.86 s vs. 123.95 ± 1.26 s, $p < 0.001$; 240.91 ± 3.21 s vs. 223.21 ± 2.54 s, $p < 0.001$. and 457.63 ± 2.39 s vs. 420.07 ± 4.60 s, $p < 0.001$, respectively) (Figure 2).

We also studied the dynamics of the contraction strength of m. tibialis anterior during continuous stimulation presented as a function: the percentage of F_{max} versus time (Figure 3).

The decline of the strength contraction was analyzed every 25 seconds from the beginning of the electrical stimulation until the 450th second of this stimulation. At 100 seconds, for example, the strength of the induced contraction was 94.04 ± 0.62% of F_{max} in the Cr-group, and 91.03 ± 0.56% in the K-group, $p < 0.01$; at 200 seconds – 74.74 ± 0.92% of F_{max} in the Cr-group, and 70.37 ± 0.68% in the K-group, $p < 0.01$; at 300 seconds – 53.90 ± 0.73% of F_{max} in the Cr-group, and 50.14 ± 0.85% in the K-group, $p < 0.01$; and at 400 seconds – 32.34 ± 0.86% of F_{max} in the Cr-group, and 26.34 ± 0.58% in the K-group, $p < 0.001$.

Because we used a mixed anaerobic-aerobic training regimen of the experimental animals, we were also interested in the aerobic capacity of the training muscles, which consist of type I muscle fibers. We assessed the activity of some oxidative enzymes in m. soleus such as

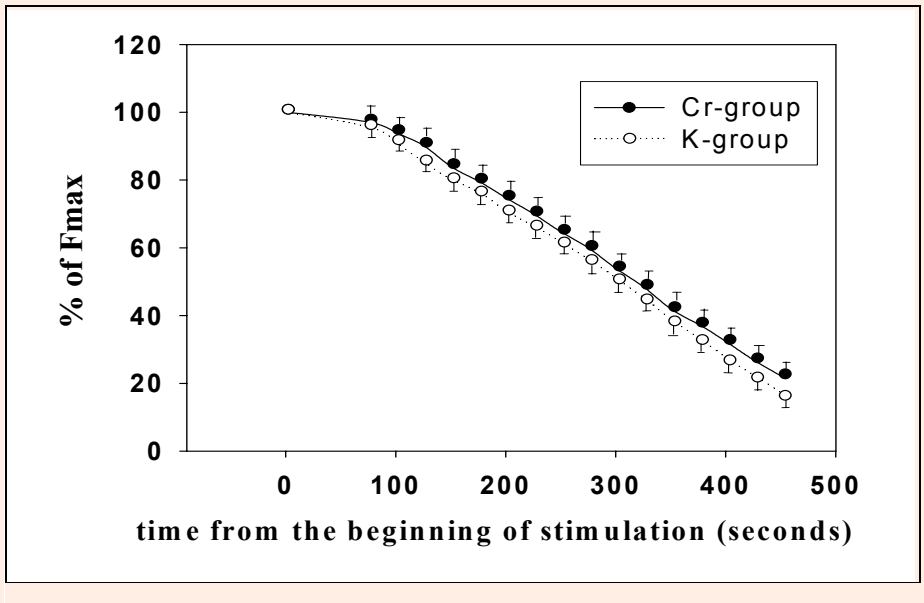

Figure 3. Changes in the strength of muscle contraction (as percentage of Fmax) during the whole time of contraction. $P < 0.001$ between the groups for all measurments.

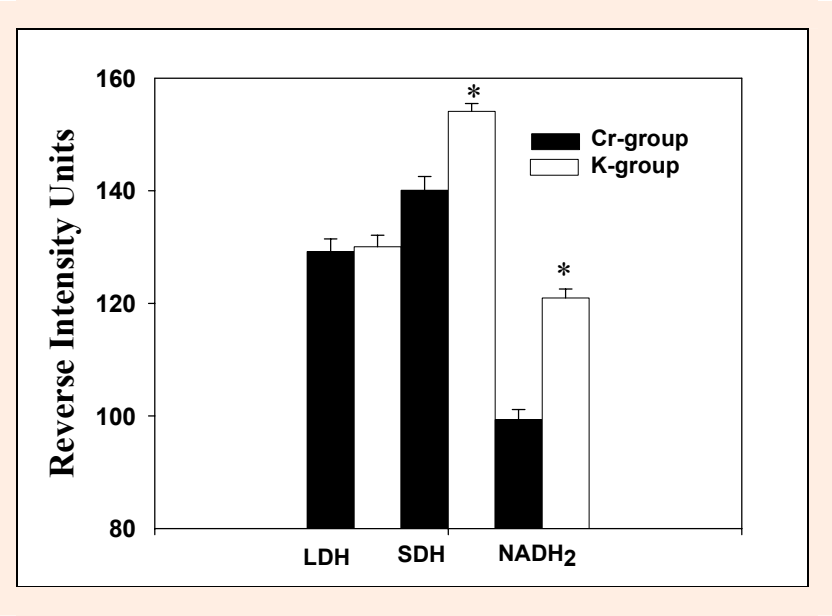

Figure 4. Intensity of enzyme expression in m.soleus at the end. * $p < 0.001$.

LDH, SDH, and $NADH_2$-cytochrome-c-reductase. We found higher activity of the selected oxidative enzymes in the Cr-group than those in the controls (note that data are presented in Reverse Intensity Units which means lower value – higher enzyme activity). The SDH and $NADH_2$-cytochrome-c-reductase showed higher activity in the Cr-group ($p < 0.001$) (Figure 4).

No differences in the studied blood hematological and biochemical variables were found between the groups except in the HCT values. It was lower in the Cr-group than in the K-group (0.419 ± 0.006 $l \cdot l^{-1}$ vs. 0.440 ± 0.007 $l \cdot l^{-1}$, $p < 0.05$). All values were within the physiological ranges for laboratory rats.

Discussion

Dietary supplementation of creatine and nutritional formulations containing creatine have become the most popular nutritional strategy employed by resistance-trained athletes to promote gains of strength (Kreider, 1995). Creatine supplementation has been reported to increase single effort and/or repetitive sprint capacity (Dawson et al., 1995; Earnest et al., 1995; Kreider et al., 1998).

Muscle cells generate mechanical work from an energy liberating chemical reaction - ATP is split into ADP and P (phosphate). ATP can be used by muscle cells very quickly, but there is only an extremely limited supply - usually only enough for a few seconds of high intensity work. When the ATP is gone, work stops. Fortunately, the body has several ways to convert ADP back to ATP. The fastest method is to move the phosphate group off of phosphocreatine and onto ADP. This yields ATP, which is immediately available for muscular work, and creatine. There is enough phosphocreatine to keep ATP levels up for several more seconds. So at this point we have moved from 2 - 3 seconds of all-out work (ATP) to almost 10 seconds (ATP + creatine). The body can recharge creatine back to phosphocreatine, but this takes time (approximately 30 - 60 seconds). This ATP + creatine system makes up the fastest component of the anaerobic system, and is most used by power athletes.

Aerobic endurance athletes, such as distance runners and triathletes, represent a much different picture from power athletes. Their levels of ATP and phosphocreatine do not change during exercise because ATP is generated at the same rate it is used – a *pay as you go* mechanism. Aerobic generation of ATP via oxidation of glucose (and fats) is slower than by anaerobic systems, but the fuel supply is enormous. Aerobic athletes train their muscles differently, and indeed the muscle tissue itself is different from power athletes. Type I muscle fibers have a slower speed of contraction than type II fibers. Slow twitch fibers have less glycolytic capacity, but increased mitochondria, myoglobin, and aerobic enzyme pathways.

Thus, slow twitch athletes cannot generate the speed and force of their *fast twitch* colleagues, but they can do their work for a long time. So the widely accepted conclusion until now has been that the ATP-creatine system is not that important for aerobic or aerobic-anaerobic athletes.

Some recent studies have found that creatine supplementation improves performance in aerobic-anaerobic trained athletes (Chwalbinska-Moneta, 2003; Engelhardt et al., 1998). Our results are consistent with these findings – better results in the sprint-test for the Cr-group (the data indicated better anaerobic-aerobic working capacity of the Cr-group at the end of the trial as a result of the creatine monohydrate supplementation – Figure 1), and better values for the variables which characterize the contractile properties. The m. tibialis anterior of the rats of Cr-group demonstrated better adaptation and during continuous stimulation had longer time to the point of reducing the strength of contraction to F_{max}-10%, F_{max}-25%, and F_{max}-50% (Figure 2). On the other hand, the fatigue curve (Figure 3) showed higher values for the strength of contraction versus time in the Cr-group than in the controls during the whole time of continuous stimulation.

The better adaptation of the oxidative enzymes in m. soleus (Figure 4) can be explained with the fact that creatine reduces the basal rate of lactate production (Ceddia and Sweeney, 2004) and this ensures a pH optimum values for SDH and $NADH_2$-cytochrome-c-reductase activity in this type I muscle.

In addition, blood biochemical and hematological data suggest that 20 days of creatine supplementation in the doses we specified above has no side effects on the organism. This is in agreement with the findings of Poortmans and Francaux (1999).

Conclusion

The results in the present study allow us to conclude that creatine supplementation of the diet in doses of 60 mg per day (an equivalent of 20 g for humans) improves the performance results, contractile properties of the fast twitch muscles, and increases the oxidative enzyme activity of aerobic-anaerobic trained rats.

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AUTHORS BIOGRAPHY

Nickolay P. BOYADJIEV
Employment
Department of Physiology, Medical University of Plovdiv, Bulgaria
Degree
PhD
Research interests
Exercise physiology, sport supplementation, resistance training, exercise endurance
E-mail: boyad@plov.net

Dobrin I. POPOV
Employment
Department of Physiology, Medical University of Plovdiv, Bulgaria
Degree
MSc
Research interests
Exercise physiology, sport supplementation, resistance training, exercise endurance
E-mail: drpopov@abv.bg

Slavi DELCHEV
Employment
Department of Anatomy, Histology and Embryology, Medical University of Plovdiv, Bulgaria
Degree
MSc
Research interests
Muscle and heart structural changes in resistance and endurance training,
E-mail: msdel@abv.bg

Key points

- The creatine monohydrate supplementation of the rats diet improves their exercise performance after 20 days administration in a dose of 60 mg per day on the background of a mixed (aerobic-anaerobic) exercise training.
- The creatine supplemented rats demonstrate better contractile properties of m. tibialis anterior which muscle contains predominantly types IIA and IIB muscle fibers.
- The soleus muscle (type I muscle fibers) demonstrates a higher oxidative capacity as a result of 20-days creatine supplementation.

✉ **Prof. Nickolay Boyadjiev, MD, PhD**
Department of Physiology, Medical University, 15-a Vassil Aprilov Blvd., 4000 Plovdiv, BULGARIA