Influence of the short and long term supplementation of creatine on the plasmatic concentrations of glucose and lactate in Wistar rats*

Renato Aparecido Souza, Roberto Mussoline dos Santos, Rodrigo Aléxis Lazo Osório, José Carlos Cogo, Antônio Carlos Guimarães Prianti Júnior, Rodrigo Álvaro Brandão Lopes Martins[1] and Wellington Ribeiro

ABSTRACT

Recent studies suggest that the creatine supplementation can interfere with glucose uptake and lactate production during the physical activity. The aim of this study was to investigate the effects of the short-term (5 $g.kg^{-1}$ for 1 week) and long-term (1 $g.kg^{-1}$ for 8 weeks) creatine supplementation on the plasmatic concentrations of glucose and lactate of sedentary and exercised (swimming to 80% of the tolerated maximum load) rats. Seventy two male Wistar rats (240 ± 10 g) were used and divided equally in 4 experimental groups (n = 18): CON – sedentary rats without supplementation; NAT – exercised rats without supplementation; CRE – sedentary rats with supplementation; CRE + NAT – exercised rats with supplementation. The blood samples were obtained weekly before and after the maximum load test. Before the maximum load test, except for the group CRE-NAT (3-5 weeks), that presented lower level of plasma glucose concentration in relationship the other groups, all the other results were similar among the experimental groups. After the maximum load test, all of the experimental groups presented reduction of the plasma glucose concentration and increase of the plasma lactate concentration. However, in relation to the glucose, this reduction was significantly ($p < 0.05$) pronounced in the groups CRE (1-4 weeks) and CRE + NAT (1-8 weeks), and in relation to the lactate, the increase was significantly ($p < 0.05$) smaller in the groups CRE (1-2 weeks) and CRE + NAT (1-8 weeks). The findings of this study suggest that the adopted regime of supplementation influenced the metabolic glycemic profile, minimized the lactate accumulation and increased the maximum load supported in the animals supplemented.

Keywords: Creatine supplementation. Ergogenic aid. Bioenergetic. Rats.

INTRODUCTION

Creatine supplementation (Cr) has been widely adopted as a nutritional strategy with the purpose to potentialize physical performance, especially by athletes[1-2]. The possible ergogenic benefits of Cr supplementation are related to its biochemical and physiological role on the skeletal muscular tissue bioenergetics[3-4]. Several mechanisms have been proposed in order to demonstrate the involvement of Cr supplementation with improved physical performance[5], among them: increase of the Creatine Phosphate indices (CP), functioning as an immediate buffer of the Adenosine Triphosphate use (ATP) during exercise; increase of the resting Cr indices in order to increase the resynthesis rate of the CP itself during and after exercise; reduction of the muscular acidity, once the CP acts consuming a H^+ in the ATP resynthesis process; increase of the Citrate Synthase activity (CS), a marker of the oxidative ability potentializing aerobic exercises; increase of the training ability and finally, increase of the muscular mass, since the CR is an osmotically active substance.

The metabolic profile and the energetic state of the muscular cells are altered according to changes in the activity degree and in the energetic substrates offer that these cells present. Some studies reveal that the Cr supplementation interferes with the glucose peripherical metabolism[6-7]. Within this context, increased rates of insulinic secretion[8], higher expression of GLUT-4 receptors[9] and increase in the glycogen intramuscular concentration[10] after supplementation are reported. It is believed that due to its hypoglycemic effect, the Cr supplementation could be benefic for the treatment of patients with type II diabetes[8].

Currently, the blood lactate has been used as an important controller of the bioenergetic conditions of the skeletal muscle[11-12]. The lactate accumulation responsible for the muscular fatigue phenomenon could be postponed due to the Cr supplementation increase of the lactic anaerobic metabolism through the energy obtained by the ATP-CP system[13]. Such characteristic could benefit long duration exercises. Nevertheless, research concerning the effects of the Cr supplementation on the biochemical markers in long duration physical activities is scarce in the literature. Therefore, the aim of this study was to evaluate the aerobic physical performance and the glucose and lactate peripherical metabolic response of rats submitted to Cr acute and chronic supplementation.

METHODS

Experimental model – 72 Wistar (*Rattus Norvegicus*) young adult male rats (240 ± 10 g), with 10-12 weeks of age, obtained from the Bem-Te-Vi farm (Paulínia/SP) were used. The animals were individually kept in polyetilene cages, in the bioterium of the Physiology and Pharmacodynamics Laboratory of the Research and development Institute of the University of the Vale do Paraíba with controlled temperature (22-25ºC), relative humidity (40-60%) and photoperiod (12 hours light-dark cycle). Moreover, all the animals had access to *ad libitum* palletized food (Labcil®) and water.

The studies had duration of eight weeks and the animals were equally divided in four experimental groups (n = 18): sedentary (CON): non-supplemented sedentary rats; exercised (NAT): non-supplemented exercised rats; sedentary Creatine (CRE): sedentary and supplemented rats; exercised Creatine (CRE + NAT): exercised and supplemented rats.

All the adopted procedures in this study were according to the laboratory animals handling and care principles recommended by

* Universidade do Vale do Paraíba – UniVap, Instituto de Pesquisa e Desenvolvimento – IP&D, Laboratório de Fisiologia e Farmacodinâmica. Av. Shishima Hifumi, 2.911 – Urbanova – 12244-400 – São José dos Campos, SP.

1. Universidade de São Paulo – USP – Instituto de Ciências Biomédicas I.

Received in 24/11/05. Final version received in 9/6/06. Approved in 11/6/06.

Correspondence to: Renato Aparecido de Souza, Rua João Nunes de Oliveira Jr., 140 – FOCH – 37550-000 – Pouso Alegre, MG. E-mail: tatosouza 2004@yahoo.com.br

the COBEA (Brazilian School of Animal Experimentation) and approved by the Ethics and Research Committee of the UniVap (Protocol # L022-2005-CEP).

Physical activity protocol – All the animals were submitted to a swimming adaptation period (30 daily minutes without load, during five consecutive days) in order to decrease factors related to the stress promoted by the swimming activity[11]. During this period, the creatine was not administered. After adaptation, the animals were individually submitted to the maximal load test (MLT)[14-15]. Load cells corresponding to 0%, 1%, 2%, 3%, and so forth, of the total body weight of the animal, were placed until its exhaustion, reaching the maximal tolerated load. The exhaustion of the animal was determined by its inability to be below the water surface for approximately eight seconds[14-15]. This test allowed the working load adjustment for the physical training at 80% of the maximal load.

The physical training at 80% of the maximal load was performed in groups of six animals due to the more vigorous exercise promotion when compared to the individual swimming[11]. Such training occurred five times a week with training daily sessions of 30 minutes and only in the NAT and CRE + NAT experimental groups. Vests with lead loads were comfortably attached to each animal's chest[11,14-15]. At the end of each experimental week, new MLT was performed for possible training load readjustments. The swimming protocol was performed in an asbestos tank with 250 liters of water kept at 35 ± 2ºC temperature.

Creatine supplementation – The supplementation was performed through an oral-esophago probe (1 mm wide; 3 cm long) adapted to a 3 ml serynge, with water as the infusion vehicle. Such procedure daily occurred after the swimming adaptation period, two hours prior to the physical training. During the first week of the experiment (loading phase), a dose of 5 g of Cr/kg of the animal's body weight was established and after the first week (maintenance phase), a dose of 1 g of Cr/kg of the animal's body weight for all the supplemented animals (CRE and CRE + NAT) was assigned[5,9,16].

Micronized creatine. (Integral Médica®), chromatographic standard degree was used in order to facilitate the absorption of the supplement and minimize any risk of contamination of the product.

Glucose and lactate analyses – The blood samples (~25 µl) were obtained through punction from the tail tip of each animal and placed in test-strips for the glucose (Blood Glucose Sensor Electrode-Medisense®) and lactate (BM-Lactate®) quantification. Afterwards, these test-strips containing the samples were immediately introduced in the MediSense portable analyzers – Q.I.D. Precision® and Accutrend® Lactate for the determination of the glucose and lactate concentrations, respectively. This procedure was performed before and after the maximal load tests.

Statistical analysis – The results were expressed as average ± standard deviation. Variance analysis (ANOVA) 4 x 4 was used for repeated measurements among the experimental groups in the different experimental periods. The *post hoc* test by Tukey for multiple comparisons was applied for the identification of the specific differences in the variables in which the F indices found were higher than the established statistical significance criterion (p £ 0,05).

RESULTS

Total body weight

The total body mass average (g) of the animals at the end of the first, fourth and eighth weeks is represented in figure 1. A statistically significant increase (p < 0,05) of body mass was observed after the first week, when the CRE-1 (294 ± 10) and CRE + NAT-1 (292 ± 13) groups are compared *versus* the CON-1 (273 ± 11) and NAT-1 (270 ± 12) groups. Similar increase was obtained after the fourth week when compared with the CRE- 4 (361 ± 13) and CRE + NAT-4 groups (352 ± 10) *versus* the CON-4 (327 ± 12) and NAT-4 (330 ± 13) groups. After the eighth week as well, the average of the body mass of the animals in the supplemented groups (CRE-8: 455 ± 13 and CRE + NAT-8: 440 ± 12) was statistically higher (p < 0,05) in relation to the non-supplemented groups (CON-8: 410 ± 12 and NAT-8: 402 ± 11).

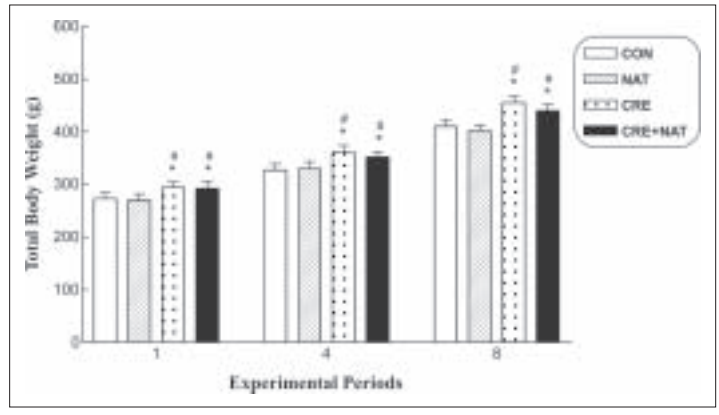

Figure 1 *– Total body weight. Results expressed as average ± standard deviation, n = 18 per experimental group, * p < 0,05* versus *CON; # p < 0,05* versus *NAT (ANOVA, followed by Tukey-Kramer test of multiple comparison).*

Tolerated maximal load

It can be observed in table 1 that the maximal load test revealed that from the first week the animals from the CRE + NAT group tolerated significant more load than the animals from the other experimental groups. Although the findings from the CRE group are higher than the ones from the non-supplemented groups (CON and NAT), these indices did not present significant differences (p > 0,05). Moreover, from the fifth week on, the animals from the NAT group supported more load than the animals from the CON and CRE groups.

TABLE 1
Tolerated maximum load

	Experimental groups			
	CON	**NAT**	**CRE**	**CRE + NAT**
After adaptation (g)	10,5 ± 2,1	10,3 ± 1,9	10,8 ± 2,6	10,2 ± 1,5
1st week (g)	10,9 ± 2,3	10,8 ± 1,9	11,7 ± 2,1	14,6 ± 2,0#
2nd week (g)	11,7 ± 2,1	11,8 ± 1,7	12,6 ± 2,4	15,6 ± 1,9#
3rd week (g)	12,6 ± 1,8	12,4 ± 2,3	13,5 ± 1,7	16,6 ± 2,4#
4th week (g)	13,4 ± 2,4	13,2 ± 2,5	14,4 ± 1,9	17,5 ± 2,3#
5th week (g)	14,1 ± 1,9	17,4 ± 1,5	15,4 ± 1,7	22,4 ± 1,7#
6th week (g)	14,8 ± 2,1	18,1 ± 1,9†	16,3 ± 2,3	23,7 ± 1,9#
7th week (g)	15,8 ± 2,2	22,9 ± 1,7*	17,3 ± 1,6	29,0 ± 2,1#
8th week (g)	16,4 ± 1,9	24,1 ± 2,1*	18,2 ± 1,9	35,2 ± 2,4#

The results were expressed as average ± standard deviation, n = 18 per experimental group, # p < 0,05 *versus* CON, NAT and CRE; † p < 0,05 *versus* CON; * p < 0,05 *versus* CON and CRE (ANOVA, followed by Tukey-Kramer test of multiple comparison).

Glucose

Table 2 presents the average indices of the plasma concentrations of pre and post-maximal load test glucose. Similarity between the results was observed before the maximal load test, except for the CRE + NAT group in the third, fourth and fifth weeks. However, all the found results in the animals from the CRE + NAT group after the maximal load test were significantly different from the CON and NAT groups. Besides that, from the fifth experimental week, the results from the CRE + NAT group were also statistically higher than the ones found in the CRE group.

TABLE 2
Pre and post maximal load test glucose

	Experimental groups			
	CON	NAT	CRE	CRE + NAT
After adaptation				
Pre-test glucose (mg/dl)	93 ± 3	95 ± 5	91 ± 6	92 ± 4
Post-test glucose (mg/dl)	77 ± 4	79 ± 3	76 ± 3	80 ± 5
1st week				
Pre-test glucose (mg/dl)	89 ± 3	89 ± 4	85 ± 2	85 ± 3
Post-test glucose (mg/dl)	77 ± 4	76 ± 2	68 ± 4#	67 ± 5#
2nd week				
Pre-test glucose (mg/dl)	88 ± 2	90 ± 5	89 ± 4	85 ± 3
Post-test glucose (mg/dl)	80 ± 4	82 ± 3	73 ± 5#	71 ± 2#
3rd week				
Pre-test glucose (mg/dl)	92 ± 3	91 ± 3	88 ± 3	75 ± 4#
Post-test glucose (mg/dl)	90 ± 5	86 ± 4	70 ± 2#	68 ± 3#
4th week				
Pre-test glucose (mg/dl)	95 ± 6	95 ± 4	90 ± 4	80 ± 2#
Post-test glucose (mg/dl)	88 ± 3	86 ± 4	74 ± 5#	71 ± 5#
5th week				
Pre-test glucose (mg/dl)	97 ± 4	93 ± 4	93 ± 3	85 ± 2#
Post-test glucose (mg/dl)	85 ± 5	80 ± 3	79 ± 4	68 ± 6#
6th week				
Pre-test glucose (mg/dl)	94 ± 5	93 ± 6	89 ± 4	89 ± 5
Post-test glucose (mg/dl)	85 ± 3	79 ± 3	79 ± 4	70 ± 2#
7th week				
Pre-test glucose (mg/dl)	89 ± 3	90 ± 5	91 ± 4	89 ± 3
Post-test glucose (mg/dl)	82 ± 2	81 ± 3	80 ± 2	72 ± 2#
8th week				
Pre-test glucose (mg/dl)	92 ± 4	94 ± 3	92 ± 5	90 ± 3
Post-test glucose (mg/dl)	85 ± 3	90 ± 3	83 ± 4	74 ± 5#

The results were expressed as average ± standard deviation, n = 18 per experimental group, # p < 0,05 *versus* other results from the same type (ANOVA, followed by Tukey-Kramer test of multiple comparison).

TABLE 3
Pre and post-test of maximal load lactate

	Experimental groups			
	CON	NAT	CRE	CRE + NAT
After adaptation				
Pre-test lactate (mmol/l)	2,5 ± 0,9	2,2 ± 0,5	2,2 ± 0,7	2,3 ± 0,6
Post-test lactate (mmol/l)	8,0 ± 0,5	7,9 ± 0,6	8,1 ± 0,5	8,2 ± 0,8
1st week				
Pre-test lactate (mmol/l)	2,8 ± 0,9	2,6 ± 0,7	2,4 ± 0,9	2,6 ± 1,0
Post-test lactate (mmol/l)	8,0 ± 0,8	8,2 ± 0,6	6,8 ± 0,8#	6,4 ± 0,7#
2nd week				
Pre-test lactate (mmol/l)	2,5 ± 0,8	2,4 ± 1,0	2,3 ± 0,7	2,0 ± 0,9
Post-test lactate (mmol/l)	8,2 ± 0,9	8,1 ± 0,8	7,1 ± 0,6#	6,8 ± 0,8#
3rd week				
Pre-test lactate (mmol/l)	2,8 ± 0,9	3,1 ± 0,7	2,6 ± 0,9	2,1 ± 0,8
Post-test lactate (mmol/l)	7,7 ± 0,7	7,9 ± 0,8	7,3 ± 0,7	6,5 ± 1,0#
4th week				
Pre-test lactate (mmol/l)	2,5 ± 0,7	3,0 ± 0,6	2,6 ± 0,5	2,4 ± 0,7
Post-test lactate (mmol/l)	8,5 ± 0,8	8,0 ± 0,9	7,9 ± 0,8	6,4 ± 0,7#
5th week				
Pre-test lactate (mmol/l)	2,7 ± 1,0	2,8 ± 0,7	2,5 ± 1,0	2,5 ± 0,6
Post-test lactate (mmol/l)	8,3 ± 0,6	7,9 ± 0,8	8,3 ± 0,6	6,8 ± 0,9#
6th week				
Pre-test lactate (mmol/l)	2,7 ± 0,9	3,0 ± 0,7	2,6 ± 0,7	2,7 ± 0,9
Post-test lactate (mmol/l)	8,5 ± 0,7	7,7 ± 0,9*	8,7 ± 06	6,3 ± 0,9#
7th week				
Pre-test lactate (mmol/l)	2,5 ± 0,8	2,7 ± 0,8	2,4 ± 0,6	2,7 ± 0,8
Post-test lactate (mmol/l)	8,4 ± 0,9	7,0 ± 0,8*	8,3 ± 0,8	5,7 ± 1,0#
8th week				
Pre-test lactate (mmol/l)	3,1 ± 0,7	3,0 ± 0,7	2,8 ± 0,9	2,6 ± 0,6
Post-test lactate (mmol/l)	8,5 ± 0,7	6,9 ± 0,6*	8,4 ± 0,9	5,7 ± 1,0#

The results were expressed as average ± standard deviation, n = 18 per experimental group, # p < 0,05 *versus* other results from the same type; * p < 0,05 *versus* CON and CRE (ANOVA, followed by Tukey-Kramer test of multiple comparison).

The CRE group presented different indices (p < 0.05) in relation to the non-supplemented groups, only in the four first weeks of the study. The post-test indices obtained in the CRE group in that period, were similar to the ones obtained in the CRE + NAT group.

Lactate

The average indices of the lactate plasma concentrations of the pre and post-maximal load test are demonstrated in table 3. It was observed that after the two first experimental weeks, the CRE and CRE + NAT supplemented groups presented significant lower lactate indices after the maximal load test in relation to the non-supplemented groups. After this period, the results found in the animals from the CRE + NAT group, after the maximal load test were also lower in relation to the CRE group. Moreover, from the sixth week on, the NAT group presented results that significantly differed from the results obtained in the CON and CRE groups after the maximal load test.

DISCUSSION

The human studies involving the Cr supplementation are usually divided in two phases: (1) initial phase with supplementation of high Cr doses (20 to 30 g daily) during 5 to 7 days (Loading Phase), immediately followed by (2) a maintenance phase, with small Cr doses of 1/5 of the initial dose (4 to 6 g daily), during several weeks. Nonetheless, since rats were used in this study and the basal metabolism rate, conversion and assimilation of organic combinations are much more intense in these animals, an extrapolation of Cr doses was made necessary for the real activity and metabolic need of the studied population. The choice of the adopted regimen for the Cr supplementation dosage in this study was made from work conducted with rats[5,9,16]. These studies observed that the 5 g and 1 g doses of Cr for each kilogram of the animal's body mass promoted ergogenic effects associated with intramuscular increases of Cr, equivalent to the ones found in humans in the loading and maintenance phases, respectively.

The literature presents countless works concerning the potential effect of the Cr over the anaerobic metabolism, especially for the bioenergetic characteristic of this compound[1-3,5]. Nevertheless, few studies discuss the effects of Cr supplementation over the aerobic metabolic resistence. In this study, the interest was exactly to evaluate the aerobic aspect, since it is little understood. Therefore, the working load for the physical training was individually adjusted to 80% of the tolerated load during the maximal load test. In this training level, the animals would perform a physical activity predominantly aerobic[17]. Adaptative changes associated with the aerobic training may be observed through alterations in dimension and in number of mitochondria of muscular cells, which favor the ATP production aerobically and benefit long duration exercises[18]. In our study, it was observed through the evaluation of the maximal load tolerated; that significant changes related to training (NAT group) occurred from the sixth experimental week. Moreover, it was observed that the Cr supplementation associated with physical training could benefit the aerobic performance from the very first week of this association. These findings admit the possibility of Cr being ergogenic as well in aerobic activities, as previously reported in the literature[5,13]. Yet there seems to be a need for interaction between physical activity and Cr supplementation for more effective gains in performance[1-2].

In addition to to anaerobic energetic buffer generated by the Cr supplementation, it has been proposed that the Cr and the CP act as messenger molecules between the mitochondria and the sub-

cellular sites of ATP production and hydrolysis, and thus may help aerobic activities[3-4]. In the mitochondrial site, the synthetized ATP enters the intermembrane space where part of it is used by the Mi-CK (Mitochondrial Creatine Kinase) for the formation of ADP and CP. The resulting ADP is hence favorably situated to be taken by a translocase to the interior of the mitochondrial matrix in the exchange for the ATP of the matrix. The resulting CP, contrary to the ADP, spread up to the myofibrillar M band, where it locally serves for ATP replacement, having the MM-CK (Myofibrillar Creatine Kinase) as catalysis agent. The resulting Cr returns to the mitochondrial intramembrane space in order to continue the cycle[3-4]. The increase of the Cr pool generated by its supplementation would favor the perpetuation of such ATP formation cycle and explain the better performance and load tolerance in the supplemented animals.

A possible fundamentation for the Cr supplementation in aerobic exercises is related with the Cr aid in buffering the ADP increases[19]. It has been reported that the ADP considerable increases act an inhibitors on the cellular ATPases, resulting in reduction in the cycle of the copling of the crossed bridges of the muscular ligands[19]. In the sarcomeres in which large quantities of ATP are hydrolyzed, the immediate ADP rephosphorilization by the MM-CK, when in supplementation, keeps a low ADP concentration avoiding hence the inactivation of ATPases myosins and not blocking the rapid generation of ATP[3-4,19].

During the occurrence of the ATP hydrolysis in the muscular contraction, protons are released (H^+). It has been suggested that the hydrogen ions increase (and concomitant decrease of the pH) during intense exercise contributes to fatigue. The ATP resynthesis by the CP occurs through the consumption of an H^+. Consequently, increasing the cellular ability of immediately phosphorilize the ADP and buffering the H^+ may serve to increase long duration physical performance. Once again the Cr supplementation could contribute to such process[20].

Some authors observed that the Cr supplementation may modify the utilization and formation of energetic substrates, such as glucose and lactate, and possibly improve physical performance during extended exercises which preferably use aerobic metabolism[1-2,5,20]. Studies which observe the glucose utilization concomitantly with lactate generation during high intensity physical activity in populations supplemented with creatine is unknown for us. Creatine is a compound which directly interferes on the muscular energetic metabolism. In a recent study, the influence of the Cr supplementation on the glucose metabolism and the lactate formation was characterized in detail[21]. It was observed that the Cr supplementation increased the expression of the Cr receptor (CT-1) and the glucose receptors (GLUT-4). Therefore, the intramuscular glycogen and Cr content was increased. The mechanism through which the high cellular concentrations of Cr and CP attenuates the Lactate Dehydrogenase (LDH) activity, decreasing thus the lactate formation, is still unknown. Besides that, the glucose stored as glycogen was preferably used via aerobic glycolysis (mitochondrial oxidation), once the Citrate Sintase (CS) activity, an aerobic ability marker, was increased.

It was also observed in our study that the Cr supplementation interfered with the glucose and lactate peripheric response. However, we acknowledge that we may simply interfere over the occurrence of lactate formation alterations and glucose consumption without reaching any conclusion about how this fact occurred. Concerning glucose, reduction in the plasmatic indices of this nutrient was observed after the maximal load test in all experimental groups. The literature reports that during long term and high intensity physical activity, the plasmatic glucose substantially contributes as energetic substrate and thus its blood concentration may be reduced[22]. Nevertheless, only the animals from the CRE + NAT group presented significant plasmatic glucose reductions after the maximal load test during the entire study. Such finding is attributed to the fact that the Cr supplementation may stimulate the insulin secretion[8,22] and increase the GLUT-4 receptors expression[9,22], both effects are hypoglicemiants. It is interesting to point that the animals that were only supplemented (CRE), presented lower glucose concentrations after the maximal load test up to the fourth week of the study. It has been proposed that the long term Cr supplementation may induce the down regulation mechanism for the CT-1 of Cr[23]. Such episode could prevent the additional inflow of this compound for the muscular cells and explain the lack of significant reduction of the plasmatic glucose from the fifth experimental week. Conversely, the physical activity itself, a factor not present in this group (CRE), could stimulate the expression of the GLUT-4 receptors and contribute to the hypoglicemiant effects[24]. Yet, since the NAT group did not present hypoglicemiant alterations, further studies should be conducted in order to better evaluate different physical activity intensity and frequency. Isolatedely, it did not promote the decrease in the glucose concentrations after the maximal load test.

The blood lactate accumulation has been described as one of the factors responsible for muscular fatigue and therefore limitator of the long term physical performance[25]. Concerning the lactate plasmatic concentrations, it is observed that the Cr supplementation associated with physical exercise reduced the lactate accumulation. The Cr supplementation increases the energy obtaintion system of the phosphagens and decreases the need for anaerobic glycolysis use, which generates lactate[1-3]. Moreover, as previously described, an LDH inhibition seems to occur, which would deviate the glucose oxidation for the aerobic metabolism[21]. Such fact associated with the renewal ATP cycle increase by the mitochondria after Cr supplementation justifies a lower use of the anaerobic glycolysis and consequently lower lactate production[3-4].

Thus, we conclude that the Cr supplementation associated with regular long duration and intensity physical exercise could benefit the performance of physical activities predominantly aerobic and decrease the accumulation of blood lactate, postponing the muscular fatigue appearance and favoring recovery after physical exertion.

All the authors declared there is not any potential conflict of interests regarding this article.

REFERENCES

1. Bemben MG, Lamont HS. Creatine supplementation and exercise performance: recent findings. Sports Med. 2005;35:107-25.
2. Kreider RB. Effects of creatine supplementation on performance and training adaptations. Mol Cell Biochem. 2003;244:89-94.
3. Greenhaff PL. The nutritional biochemistry of creatine. Journal of Nutritional Biochemistry. 1997;11:610-8.
4. Walker JB. Creatine: biosynthesis, regulation and function. Adv Enzymol Relat Areas Mol Biol. 1979;50:177-242.
5. Brannon TA, Adams GR, Gonniff CL, Baldwin KM. Effects of creatine loading and training on running performance and biochemical properties of rat skeletal muscle. Med Sci Sports Exerc. 1997;29:489-95.
6. Ferrante RJ, Andreassen OA, Jenkins BD, Dedeoglu A, Kuemmerle S, Kubilus JK, et al. Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. J Neurosci. 2000;15:4389-97.
7. Robinson TM, Sewell DA, Hultman E, Greenhaff PL. Role of submaximal exercise in promoting creatine and glycogen accumulation in human skeletal muscle. J Appl Physiol. 1999;87:598-604.
8. Gempel K, Brdizyczka D, Kaddurah-Daouk R, Wallimann T, Kaufhold P, Gerbitz KD. The creatine analog cyclocreatine increases insulin secretion in INS-1 cells via K+ channel independent mechanism. Diabetologia. 1996;39:31-7.
9. Op'T Eijnde B, Urso B, Richter EA, Greenhaff PL, Hespel P. Effect of oral creatine supplementation on human muscle GLUT-4 protein content after immobilization. Diabetes. 2001;50:18-23.
10. Young JC, Young RE. The effect of creatine supplementation on glucose uptake in rat skeletal muscle. Life Sci. 2002;71:1731-7.

11. Voltarelli FA, Gobatto CA, Mello MAR. Determination of anaerobic threshold in rats using the lactate minimum test. Braz J Med Biol Res. 2002;35:1389-94.
12. Brooks GA. Intra- and extracellular lactate shuttles. Med Sci Sports Exerc. 2000;32: 790-9.
13. Stroud MA, Holliman D, Bell D, Green AL, Macdonald I, Greenhaff PL. Effect of oral creatine supplementation on respiratory gas exchange and blood lactate accumulation during steady-state incremental treadmill exercise and recovery in man. Clin Sci (Colch). 1994;87:707-10.
14. Osorio RA, Silveira VL, Maldjian S, Morales A, Christofani JS, Russo AK, et al. Swimming of pregnant rats at different water temperatures. Comp Biochem Physiol A Mol Integr Physiol. 2003;135:605-11.
15. Osorio RA, Christofani JS, D'Almeida V, Russo AK, Picarro IC. Reactive oxygen species in pregnant rats: effects of exercise and thermal stress. Comp Biochem Physiol C Pharmacol Toxicol. 2003;135:89-95.
16. Tarnopolky MA, Bourgeois JM, Snow R, Keys S, Roy BD, Kwieeien JM, et al. Histological assessment of intermediate and long-term creatine monohydrate supplementation in mice and rats. Am J Physiol Regul Integr Comp Physiol. 2003;285:762-9.
17. Sampaio-Barros MM, Farias-Silva E, Grassi-Kassisse DM, Spadari-Bratfisch RC. Effect of swimming session duration and repetition on metabolic markers in rats. Stress. 2003;6:127-32.
18. Bizeau ME, Willis WT, Hazel JR. Differential responses to endurance training in subsarcolemmal and intermyofibrillar mitochondria. J Appl Physiol. 1998;85:1279-84.
19. McMillnen J, Donovan CM, Messer JI, Willis WT. Energetic driving forces are maintained in resting rat skeletal muscle after dietary creatine supplementation. J Appl Physiol. 2001;90:62-6.
20. Vandenbergue K, Goris M, Van Hecke P, Van Leemputte M, Van Gerven L, Hespel P. Long-term creatine intake is beneficial to muscle performance during resistance training. J Appl Physiol. 1997;83:2055-63.
21. Ceddia RB, Sweeney G. Creatine supplementation increases glucose oxidation and AMPK phosphorylation and reduces lactate production in L6 rat skeletal muscle cells. J Physiol. 2004;555:409-21.
22. Rooney K, Bryson J, Phuyal J, Denyer G, Caterson I, Thompson C. Creatine supplementation alters insulin secretion and glucose homeostasis in vivo. Metabolism. 2002;51:518-22.
23. Guerrero-Ontiveros ML, Wallimann T. Creatine supplementation in health and disease. Effects of chronic creatine ingestion in vivo: down regulation of the expression of creatine transporter isoforms in skeletal muscle. Mol Cell Biochem. 1998; 184:427-37.
24. Kraniou GN, Cameron-Smith D, Hargreaves M. Effect of short-term training on GLUT-4 mRNA and protein expression in human skeletal muscle. Exp Physiol. 2004;89:559-63.
25. Khanna GL, Manna I. Supplementary effect of carbohydrate-electrolyte drink on sports performance, lactate removal & cardiovascular response of athletes. Indian J Med Res. 2005;121:665-9.