174 - CREATINE SUPPLEMENTATION AND PHYSICAL TRAINING: EFFECTS ON MORPHO-FUNCTIONAL PARAMETERS.

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INTRODUCTION:

In the last years, creatine has received a considerable attention in scientific researches and has been widely used as an ergogenic resource. Several studies (OP'TEIJNDE et al., 2000; ROBINSON et al., 2000; McMILLEN et al., 2001) have reported the beneficial effects of creatine supplementation combined with physical exercise, both in animals and in human beings. Creatine is used to restore, correct or modify the phyisiological functions of individuals who practice different types of sports. Therefore, its daily dose, administration period and performance are important for the effectiveness and safety of the oral creatine supplementation (BENZI,2000).

The overload of creatine monohydrate can cause an increase in the phosphocreatine content, providing more substratum for the phosphagenic system during intense muscular contraction (FEBBRAIO et al., 1995).This substratum provides a better performance in physical activities that use mainly this source of energy for the generation of ATP. Therefore, this supplementation can lead to a significant increase in the muscular mass. On the other hand, some studies have questioned if the long term use of creatine may lead to damages to the users' health due to increased cases of cramps, muscular and renal damage, intolerance to heat (TAES et al., 2003; WATSON et al.,2006).

Our study investigated the influence of the monohydrate creatine supplementation in animals submitted to physical training for 8 weeks, assessing the renal, hepatic, muscular and fatty mass parameters.

MATERIALS AND METHODS

Animals - All aspects of the experimental protocols were approved by the Ethics Committee on Animal Experiments of the State University of Maringa. We employed 32 male Wistar rats, that were treated with water and chow *ad libitum*. The animals were given two different diets: the standard Nutrilab diet (C) and the 5% monohydrate creatine supplement diet (Cr) added to the standard diet from the fourth week of practice to the end of the protocol. **Experimental procedure** - The animal were divided into four groups: sedentary (S), sedentary with creatine supplementation (SCr), training (T) and training with creatine supplementation (TCr). The training period lasted for eight weeks. The animals ran on a treadmill 5 times a week for 60 minutes, besides high intensity training and short periods on a treadmill, according to a protocol by DUFLONT & MICHELINI (1997), adapted by NEGRÃO et al. (1992). After finishing the training period, the animals were sacrificed in resting condition. ***Tissue collecting*** - The animals were anestethized with sodium Pentobarbital (Hypinol® 3%, 4 mg/100g p.c., i.p.). Then, a mediane laparotomy was performed to collect the blood (4 mL) from the inferior vena cava and removal of several tissues (liver, periepididymal and retroperitoneal fat pads, soleus and gastrocnemius muscles). I***solation of adipocytes and morphometric analysis*** - The adipocytes were isolated in accordance with RODBELL (1964), with some modifications to adapt the method to our lab conditions. Then, the diameters of 100 adipocytes per rat were randomly measured through a capture and image analysis system (Image-Pro Plus 4.5 Media Cibernetics). From this measurement, we made some calculation to determine the average diameter of the adipocytes per group. ***Biochemical dosages***: The concentration of plasma glucose was determined by glucose-oxidase enzymatic method, specific for glucose quantification (LOTT & TURNER, 1975). We used the Glucose Enzymatic Kit (Cat. 234, Analisa). The creatinine concentration was quantified by the colorimeter method (Creatinine Colorimetric, Kit CELM®), in the animals' serum and urine. The serum albumin and the bilirubin were enzymaticaly determined both through specific kits produced by Goldanalisa Diognóstica®. The protein dosage was calculated, based on the urine collection in 24 hours, by the proteinuria kit (Goldanalisa®). ***Determination of hepatic and muscular glycogen contents*** - Samples of the frozen tissues were digested in a solution of KOH 30% (500 mg of tissue/2 ml of KOH), under heat. We used the SJÖRGREEN et al. (1938) method. The glycogen amount was assessed by the anthrone method, according to the HASSID & ABRAHAM (1957) technique; the final value was expressed in mg/100 mg of tissue. ***Statistical analysis*** - the statistical evaluation of the results was accomplished by the test "t" of Student for non-paired samples or "one-way analysis of variance" (ANOVA) with Newman-Keuls tests of multiple comparison, when necessary. We establish a significance level of 95% ($p < 0,05$). The statistical tests were performed by the Prism software, v.2.1 (GraphPad, USA).

RESULTS AND DISCUSSION

The animals had their weight, water consumption and food intake controlled weekly and the results are expressed in Table 1. Our data show that there was no significant difference among the groups regarding the water consumption and food intake. The only alteration in the food intake occurred when the switch from the chow without creatine to the creatine chow. As for the body weight, there was no significant difference, considering the differences between the final and initial weight means, between the sedentary and training groups.

The renal and hepatic functional parameters were assessed through the plasmatic dosages as shown in table 02. We did not observe significant changes in the hepatic function, bilirubin and albumin markers, in any of the experimental groups. As for the renal function, although the creatine supplemented animals presented a significant increase when compared to the controls, these values are still within the normality range for creatinine.

TABLE 01. .Effect of the 4-week-creatine supplementation in sedentary animals or in 8-week-treadmill-training animals

	Body Weight (g)		Food intake (g/day)		Water consumption (ml/day)	
	initial	Final	Initial	final	initial	Final
S (8)	271,5±8,5	437,0±16,4	29,7± 1,6	28,9± 0,98	57,5±1,1	47,5±1,4
SCr (8)	274,2±6,5	412±11,7	27,2± 0,87	27,6±0,93	53,7±0,78	45,0±1,2
T (8)	273,75±4,3	399±13,7	28,4± 1,5	27,4±1,1	56,2±0,97	48,7±1,7
TCr (8)	274,0±3,0	400,5±11,0	26,6±0,89	25,7±1,2	57,5±1,3	48,7±0,78

(n) = number of animals per group. Values express means ± SE.

TABLE 02. Renal and hepatic function index in sedentary and trained animals after 4 weeks of creatine supplementation.

	Sedentary (S)		Trained (T)	
	Controls (n=8)	Creatine (n=8)	Controls (n=8)	Creatine (n=8)
Serum Creatinine (mg/dl)	0,40±0,05	1,35±0,10[a]	0,42±0,04	1,60±0,13[a]
Creatinine in Urine (mg/dl)	1,13±0,16	3,29±1,04[a]	1,26±0,26	2,69±0,46[a]
Protein urinary excretion 24 h (mg/24h)	5,36±1,06	4,42±0,81	5,53±1,19	7,87±1,35
Serum Albumin (mg/dl)	1,63±0,28	2,0±0,02	1,98±0,11	1,80±0,16
Albumin in urine (mg/dl)	0,12±0,03	0,08±0,01	0,11±0,03	0,11±0,01
Bilirubin (umol/L)	10 ±2	12 ±6	12 ±6	10 ±2

[a] P<0,05, supplemented animals when compared to their controls.

The glycogen concentration was evaluated in the hepatic area and in two muscle groups. The percentage of fast and slow contraction fibers were differentiated (table 03). The physical training alone did not cause any change in the glycogen concentration in any of the analyzed tissues. On the other hand, the monohydrate creatine supplementation alone or associated with physical training caused an increase in the glycogen amount in the liver and in the soleus. The supplementation only had effect in the gastrocnemius muscle when associated with physical exercise.

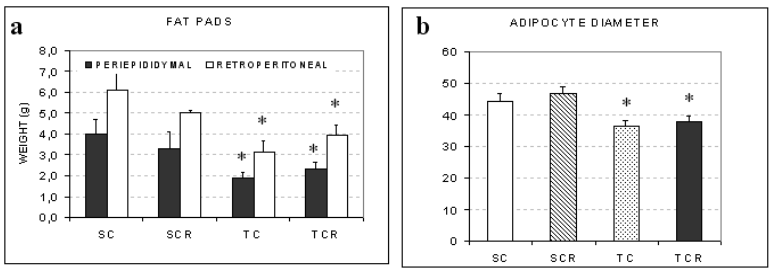
Tabela 3. Glycogen concentration values in the liver and muscles of animals in the different experiment groups in mmol/Kg^{-1}.

	Hepatic	Soleos	Gastrocnemius
S (8)	126 ± 2,4	116 ± 4,2	168 ± 7,5
SCr (8)	146 ± 3,1*	135 ± 5,1*	187 ± 6,5
T (8)	131 ± 4,2	111 ± 4,5	192 ± 10,5
TCr (8)	153 ± 6,7*	152 ± 8,5*#	227 ± 9,5**

(n) = number of animals per group, values represent Mean ± SE. * p < 0,05 for control animals # p<0,05 for sedentary controls. ** p < 0,05 for sedentary animals. # p< 0,01 for groups S and SCr.

We removed and weighed the retroperitoneal and periepididimal fat pads in order to verify any alterations in the body composition due to the creatine supplementation (graph 1a). We observed that the physical training caused a significant reduction in the fat pad weight; however, the creatine did not change this parameter. The adipocytes in the periepididimal area were isolated with the technique described in methods and we measured the diameters (graph 1b). Also in this aspect, although the physical training has significantly reduced the size of fat cells, the creatine exerted no effect.

The physical training did not lead to changes in the body weight but there was a significant reduction in the adipose mass of the trained animals. However, the creatine supplementation did not cause any change in these parameters. In many cases, the increase of lean mass is associated to the reduction of fat mass, due to a higher basal energy burning. Some studies have shown an increase in the lean mass due to the creatine supplementation (FERREIRA et al., 2005).



Graph 1. Analisys of fat tissue in the experiment groups **a**) Weight of retroperitoneal and periepididymal fat pads **b)** Isolated adipocytes diameter. The values show the mean SE of 8 animals per group. * p < 0,05 for sedentary animals.

CONCLUSIONS

Our study showed that the creatine supplementation did not cause any changes in the functional parameters in the renal and hepatic tissues, but it improved the glycogen amounts in the muscles and liver. There was no change in the fat tissue due to the use o creatine. However, the physical training significantly reduced the adipocytes diameter.

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CREATINE SUPPLEMENTATION AND PHYSICAL TRAINING: EFFECTS ON THE MORPHO-FUNCTIONAL PARAMETERS

Abstract

The objective of this study was to assess the effects of the creatine supplementation on the hepatic, renal and muscular functional parameters and the morphologic parameters of the fat tissue. **METHODS:** Male Wistar rats were submitted to physical training on treadmill for 8 weeks and supplemented with monohydrate creatine (2g/kg of ration) for 4 weeks. The groups were divided in four groups: sedentary controls (S), creatine sedentary (SCr), training controls (T) and creatine training (TCr). At the end of the protocol, samples of blood, urine and hepatic, muscular and fat tissues were collected. The biochemical dosages were accomplished by colorimeter methods and the adipocytes isolation by the method of Rodbell. **RESULTS:** There was no difference in the body weight, food intake and water consumption among the groups. Although the creatinine serum values were higher in the supplemented animals (SCr 1,35 ± 0,10 mg/dl and TCr 1,60 ± 0,13 mg/dl) when compared to the controls (S 0,40 ± 0,05 and T 0,42 ± 0,04 mg/dl), these values are considered normal. The albumin, proteins and bilirubin values were not different among the groups. The glycogen values were higher in the supplemented animals when compared to the controls. The fat pads weight and the cellular diameters were smaller in the training animals but the creatine did not have any effect on this parameter. **CONCLUSION:** The creatine supplementation did not harm the renal and hepatic function, improved the amount of glycogen in the muscles and liver, but did not exert any effect on the fat tissue. However, the physical training significantly reduced the adipocytes diameter.

Keywords: creatine supplementation, physical training, morpho-physiology.

SUPPLÉMENTATION DE CRÉATINE ET FORMATION PHYSIQUE: L'EFFETS SUR PARAMÈTRES MORPHOLOGIQUES ET FONCTIONNELS

Résumé

L'objectif de cette étude était d'évaluer les effets de la supplémentation de créatine sur les paramètres fonctionnels hépatiques, rénaux et musculaires et les paramètres morphologiques du gros tissu. MÉTHODES : Des rats Wistar ont été soumis à la preparation physique sur le tapis roulant pendant 8 semaines et supplémentés avec du monohydrate de la créatine (2g/kg de ration) pendant 4 semaines. Les animaux ont été divisés dans quatre groupes : contrôles sédentaires (s), créatine sédentaires (SCr), contrôles exerçantes (t) et créatine exerçantes (TCr). À la fin du protocole, des échantillons de sang, l'urine et les tissus hépatiques, musculaires et gros ont été rassemblés. Les dosages biochimiques ont été accomplis par des méthodes de colorimètre et l'isolement d'adipocytes par la méthode de Rodbell. RÉSULTATS : Il n'y avait aucune différence dans le poids corporel, l'ingestion de nourriture et la consommation de l'eau parmi les groupes. Les valeurs de sérum de créatinine aient été plus hautes chez les animaux supplémentés (SCr 1.35 ± 0.10 mg/dl et TCr 1.60 ± 0.13 mg/dl) une fois comparé aux comtrôles (S 0.40 ± 0.05 et T 0.42 ± 0.04 mg/dl), mais ces valeurs sont considérées normales. Les valeurs de l'albumine, des protéines et bilirubine n'étaient pas différents parmi les groupes. Les valeurs en glycogène étaient plus hauts chez les animaux supplémenté une fois comparés aux comtrôles. Les grosses garnitures pèsent et les diamètres cellulaires étaient plus petits chez les animaux de formation, mais la créatine n'a eu aucun effet sur ce paramètre. CONCLUSION : La supplémentation de créatine n'a pas nui à la fonction rénale et hépatique; elle a amélioré la quantité de glycogène dans les muscles et le foie, mais n'a pas exercé aucunne effet sur le gros tissu. Cependant, la formation physique a réduit de manière significative le diamètre d'adipocytes.

Mots-clés : supplémentation de créatine, formation physique, morphologique physiologie.

SUPLEMENTACIÓN CON CREATINA Y ENTRENAMIENTO FÍSICO: EFECTOS SOBRE LOS PARÁMETROS MORFOLÓGICOS Y FUNCIONALES

Resumen

El objetivo de este estudio se evaluaron los efectos de la suplementación con creatina en los parámetros funcionales hepáticos, renales y musculares y los parámetros morfológicos del tejido adiposo. MÉTODOS: Las ratas Wistar fueron sometidas al entrenamiento físico en la rueda de ardilla por 8 semanas y suplidas con el monohidrato de creatina (2g/kg de la ración) por 4 semanas. Dividieron a los animales en cuatro grupos: controles sedentarios (s), creatine sedentarios (SCR), controles de entrenamientos (t) y entrenamiento de la creatina (TCr). En el final del protocolo, las muestras de la sangre, la orina y los tejidos hepáticos, musculares y adiposos fueron recogidos. Las dosificaciones bioquímicas fueron logradas por métodos del colorímetro y el aislamiento de los adipocitos por el método de Rodbell. RESULTADOS: No había diferencia en el peso corporal, la toma de comida y la consumición del agua entre los grupos. Aunque los valores del suero de la creatinina eran más altos en los animales suplidos (SCR 1.35 ± 0.10 mg/dl y TCr 1.60 ± 0.13 mg/dl) en comparación a los controles (S 0.40 ± 0.05 y T 0.42 ± 0.04 mg/dl), estos valores son considerados normales. Los valores de la albúmina, proteínas y bilirubin no fueran diferentes entre los grupos. Los valores del glicógeno fueran más altos en los animales suplidos en comparación con los controles. El peso de los tejidos adiposos y los diámetros celulares eran más pequeños en los animales del entrenamiento pero la creatina no he tenido ningún efecto en este parámetro. CONCLUSIÓN: La suplementación con creatina no dañó la función renal y hepática; ella mejoro la cantidad de glicógeno en los músculos y hígado, sino no ejerció ningún efecto sobre el tejido adiposo. Sin embargo, el entrenamiento físico redujo perceptiblemente el diámetro de los adipocitos.

Palabras claves: suplementación de la creatina, entrenamiento físico, morfología-fisiología.

SUPLEMENTAÇÃO DE CREATINA E TREINAMENTO FÍSICO: EFEITOS EM PARÂMETROS MORFOFUNCIONAIS

Resumo

INTRODUÇÃO: O objetivo do presente estudo foi avaliar os efeitos da suplementação com creatina de parâmetros funcionais hepáticos, renais e musculares e parâmetros morfológicos do tecido adiposo. **MÉTODOS:** Ratos Wistar machos foram submetidos a treinamento físico em esteira rolante por 8 semanas e suplementados com monohidrato de creatina (2g/kg de ração) por 4 semanas, os grupos foram divididos em quatro grupos: sedentários controles (S), sedentários creatina (SCr), treinados controles (T) e treinados creatina (TCr). Ao final do protocolo foram coletadas amostras de sangue, urina e tecidos hepático, muscular e adiposo. As dosagens bioquímicas foram realizados por métodos colorimétricos e isolamento dos adipócitos pelo método de Rodbell. **RESULTADOS:** Não houve diferença no peso corporal, consumo de ração e hídrico entre os grupos. Embora os valores de creatinina sérica tenham sido maiores nos animais suplementados (SCr 1,35 ± 0,10 mg/dl e TCr 1,60 ± 0,13 mg/dl) comparados aos controles (S 0,40 ± 0,05 e T 0,42 ± 0,04 mg/dl) estes valores são considerados normais. Os valores de albumina, proteínas e bilirrubina não diferiram entre os grupos. Os valores de glicogênio foram maiores nos animais suplementados em comparação aos controles. Os peso dos coxins adiposos e os diâmetros celulares foram menores nos animais treinados mas a creatina não teve efeito sobre este parâmetro. **CONCLUSÃO**: A suplementação com creatina não prejudicou a função renal e hepática, melhorou o teor de glicogênio muscular e no fígado, mas não exerceu efeito sobre o tecido adiposo. Porém, o treinamento físico reduziu significativamente o diâmetro dos adipócitos.

Palavras-chave: suplementação de creatina, treinamento físico, morfofisiologia.