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Research article

Effects of high-dose creatine supplementation on kidney and liver responses in sedentary and exercised rats

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Abstract

This study evaluated the effects of high-dose of short-term creatine supplementation ($5g \cdot kg^{-1} \cdot day^{-1}$ to 1 week) and long-term creatine supplementation ($1g \cdot kg^{-1} \cdot day^{-1}$ to 4-8 weeks) on kidney and liver structure and function of sedentary and exercised Wistar rats (Exercise sessions consisted of swimming at 80% of maximal work load supported during 5 days per week with daily sessions of 60 minutes throughout the duration of the supplementation). Seventy-two animals (245 ± 5g) were divided into four groups (n = 18): control diet Sedentary (SED), Creatine diet Sedentary (CRE), control diet Exercised (EXE), and Creatine diet Exercised (EXECRE). Histological and blood biochemical studies were performed after one, four, and eight weeks of creatine supplementation and exercise (n = 6). No differences were found when comparing SED, EXE and EXECRE groups for kidney and liver structure and function at one, four and eight weeks. However, the CRE group showed higher levels of creatinine (1.1 ± 0.2 *vs.* 0.4 ± 0.1 $mg \cdot dl^{-1}$; $p < 0.05$), and urea (37 ± 3 *vs.* 19 ± 1 $mg \cdot dl^{-1}$; $p < 0.05$) when compared with all others groups at four and eight weeks. At eight weeks, the CRE group presented increased levels of ALT (41 ± 7 *vs.* 23 ± 7 $U \cdot L^{-1}$; $p < 0.05$), AST (89 ± 6 *vs.* 62 ± 5 $U \cdot L^{-1}$; $p < 0.05$), GGT (8.0 ± 0.9 *vs.* 3.9 ± 1.0 $U \cdot L^{-1}$; $p < 0.05$), and AP (125 ± 10 *vs.* 69 ± 9 $U \cdot L^{-1}$; $p < 0.05$) also when compared with all others groups. Moreover, the CRE group demonstrated some structural alterations indicating renal and hepatic damage at four and eight weeks, respectively. These results suggest that long-term creatine supplementation (up to 4-8 weeks) may adversely affect kidney and liver structure and function of sedentary but not of exercised rats.

Key words: Creatine monohydrate, hepatic metabolism, kidney metabolism, swimming training, sports supplements, toxicology.

Introduction

Creatine (Cr) is an organic compound synthesized mainly in the liver and kidneys from the amino acids glycine, arginine and methionine (Walker, 1979). Cr plays an important role in rapid energy provision during muscle contraction involving the transfer of N-phosphoryl group from phosphorylcreatine (PCr) to ADP to regenerate ATP through a reversible reaction catalyzed by phosphorylcreatine kinase (PCK) (Walker, 1979). Its biochemical (Greenhaff, 1997), physiological (Greenhaff, 1997), ergogenic (Bemben and Lamont, 2005) and therapeutic (Gualano et al., 2009; Vieira et al., 2007) roles have been extensively investigated. In the last 20 years, Cr has become a very popular dietary supplement (Bird, 2003; Maughan et al., 2004) but despite its widespread use, there is little evidence concerning possible side effects (Bizzarini and De Angelis, 2004; Poortmans et al., 2005).

Studies have found that Cr supplementation can increase skeletal muscle and brain total Cr and PCr concentrations, with an even greater degree of increase seen in organs with low baseline creatine content such as kidney and liver (Ipsiroglu et al., 2001) but the possible side effects of Cr supplementation, such as renal dysfunction, and hepatotoxicity are still inconclusive (Bizzarini and De Angelis, 2004). Because urea, which is one of the metabolic products of creatine metabolism, is involved in the conversion of toxic compounds such as methylamina and formaldehyde, Cr supplementation can also be expected to influence this conversion (Poortmans et al., 2005). There has been some concern regarding the potential for Cr supplementation toxicity based on two anecdotal human case reports (Pritchard and Kalra, 1998; Thorsteinsdottir, et al. 2006). However, in humans, most of the studies that have examined the potential for toxicity of Cr supplementation have not found evidence of side effects when consumed at "recommended" doses (Kreider et al., 2003; Mayhew et al., 2002; Mihic et al., 2000; Poortmans and Francaux, 1999**;** Poortmans et al., 1997; Robinson et al., 2000; Terjung et al., 2000; Waldron et al., 2002). In animals, the effects of Cr supplementation on renal and hepatic structure and function have not been well established. Whereas some studies did not report any alteration in renal and hepatic function after Cr supplementation (Taes et al., 2003; Tarnopolsky et al., 2003), others have observed that it can speed up renal and hepatic disease progression (Edmunds et al., 2001; Ferreira et al., 2005; Tarnopolsky et al., 2003).

Vieira et al. (2007) demonstrated that Cr supplementation exacerbates all cardinal features of asthma in mice with chronic allergic airway inflammation, which suggests potential side effects for asthmatic individuals. However, the same authors recently demonstrated that these pulmonary side effects are completely abolished when Cr supplementation in animals with chronic allergic airway inflammation is followed by aerobic exercise, suggesting that the exercise could to block the possible side effects of Cr supplementation (Vieira et al., 2008b).

Since supraphysiological doses of Cr supplementation could facilitate the demonstration its potential for side effects at the tissue level, in this study we have used

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histopathological and enzymatic analyses to clarify the possible protector effect of exercise during high-dose Cr supplementation. In general, renal diseases are characterized by the occurrence of morphological lesions at any degree of magnification and also by any biochemical abnormality (Gregory, 2003), although a severe renal disease can be present without clinical signs or laboratorial alterations that indicate renal insuficiency (Gregory, 2003). Therefore, plasma levels of urea and creatinine are classical markers of renal function because they represent a simple marker of glomerular filtration (Ghosh and Sil, 2007). Liver diseases have presented a broad variability in several human and animal studies, as well as in the criteria used to categorize the severity of hepatotoxicity (Nunez, 2006). Plasma levels of hepatic enzymes, such as aspartate aminotranferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP) are considered valuable for detecting toxic effects on the liver (Boada et al., 1999).

Therefore, the aim of this study was to evaluate the effects of supraphysiological doses of loading and maintenance phase protocol of Cr supplementation on the renal and hepatic structure and function in sedentary and exercised Wistar rats.

Methods

All animal care was in accordance with the guidelines of the Brazilian College for Animal Experimentation (COBEA) and was approved by Animal Research Ethics Committee of the University of Vale do Paraíba (L022-2005-CEP).

Experimental design

Seventy-two male Wistar rats (245±5g) were housed individually (22 ± 3 ºC, relative humidity 60 ± 10%, 12h/12h light/darkness); food and water was available *ad libitum*. The study was performed for 8 weeks and the animals were divided into four groups (n = 18): control diet Sedentary (SED), Creatine diet Sedentary (CRE), control diet Exercised (EXE), and Creatine diet Exercised (EXECRE). To verify the effects of supraphysiological doses of Cr loading and maintenance supplementation periods, six animals in each group were euthanized by an intracardial injection of pentobarbital sodium at the end of 1, 4, and 8 weeks.

Swimming training

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 (Osorio et al., 2003a; 2003b). During this period, the Cr was not administered. After adaptation, the animals were individually submitted to the maximum load test (MLT) (Guerrero-Ontiveros and Wallimann, 1998; Ipsiroglu et al., 2001). Load cells (lead fish sinkers) were increased at 3 min intervals by weights corresponding to 1, 2, 3%, etc. of the rat's mass. The load cells were attached to the tail of the animal until the maximal work load was reached, which was determined at the moment when the animal became exhausted (unable to surface after approximately 8-10s). 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 (Osorio et al., 2003a; 2003b). Such training occurred five times a week with training daily sessions of 60 minutes throughout the duration of the supplementation and only in the EXE and EXECRE experimental groups. The swimming protocol was performed in an asbestos tank with 250 liters of water kept at 31 ± 1 ºC temperature.

Creatine supplementation

Cr supplementation began one day after the swimming adaptation. The animals received the Cr supplementation by gavage (Micronized Creatine, Integral Médica®, Embu-Guaçu, SP, Brazil). at a dose of $5g \cdot kg^{-1} \cdot day^{-1}$ to 1 week (loading phase) and $1g \cdot kg^{-1} \cdot day^{-1}$ to 4-8 weeks after loading phase (maintenance phase). Doses were given in the afternoon and corresponded 30 min prior to exercise.

In addition, considering that a dose daily of 300mg of creatine per kilogram of body weight is routinely used in other animal studies (Brannon et al., 1997; Gagnon et al., 2002; McGuire et al., 2002; Young and Young, 2007) and is equivalent to the customary loading dose of $20g \cdot day^{-1}$ in a 70 kg person which produces maximal effects in 5 days, the Cr supplementation regimen adopted in the present study must be considered supraphysiological.

Enzymatic measurements

Forty-eight hours after the last training session and creatine administration, animals were anesthetized with ketamine ($70\ mg \cdot kg^{-1}$) and xylazine ($1\ mg \cdot kg^{-1}$). The abdominal cavity was opened and blood samples (5ml) were collected from the inferior cava vein into plastic syringes carefully avoiding the formation of bubbles. After centrifugation at 1000 rpm for 10 minutes, the plasma was collected and biochemical analysis for renal function (Urea and Creatinine), and hepatic function (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma Glutamyltransferase (GGT), Alkaline Phosphatase (AP), Albumin and Total Bilirubin, were measured spectrophotometrically following the manufacturer's manual (Hitachi®, Model U-2001) using commercial kits (Laborlab, Guarulhos, SP, Brazil) (Gregory, 2003, Vieira et al, 2008a).

Histological procedures

After blood collection, the right kidney and right hepatic lobe were rapidly removed and fixed in formalin solution (10%) for 24 hours and submitted to histological routine. The 5µm slides were stained with Hematoxiline/Eosine and photographed at 400x magnification, with a digital camera (Nikon, CoolPix) attached to an optical microscope (Nikon, Eclipse E200). For kidneys we analysed the structure of Bowman capsules, glomerular capillaries, intra-capsular spaces, renal tubules, as well as the presence of leukocytes, red cells and edema formation. For liver, we analysed the centrality of nucleus, number of hepatocytes with binucleation, congestion of central vein, leukocytes infiltration, number of kupffer cells and swell-

ing lumen by the blood cells and congestion of sinusoids (Vieira et al., 2008a).

Statistical analysis

The results were expressed as mean ± SEM. Two way variance analysis (ANOVA) 4 x 3 (group x time) was used for repeated measurements among the experimental groups in the different experimental periods. The Tukey post hoc test was applied for the identification of the specific differences in the variables. The level of significance was set at $p < 0.05$. The statistical analysis was conducted using the software SPSS 17.0 for Windows (SPSS Inc., Chicago, IL).

Results

Effects of creatine supplementation during loading phase

Figure 1 shows the plasma levels of renal (creatinine, urea) and hepatic function (ALT, AST, GGT, AP, Albumin and Total Bilirubin) markers after 1 week of Cr supplementation. No harmful effects of Cr loading supplementation ($5g \cdot kg^{-1} \cdot day^{-1}$ for 1 week) on renal and hepatic function markers were observed during this phase. Only AST presented higher values in the exercised groups (EXE and EXECRE) when compared with the sedentary groups (SED and CRE) (Figure 1D; $p < 0.05$). Additionally, renal and hepatic histological evaluation did not show any alterations for all groups.

Effects of creatine supplementation during maintenance phase

Cr supplementation during maintenance phase ($1g \cdot kg^{-1} \cdot day^{-1}$ for 4 and 8 weeks after loading phase) led to increased levels of renal function markers (creatinine and urea) (Figures 2A and B and 3A and B, respectively). However, these results were evident only in CRE group when compared with all others groups ($p < 0.05$).

After the fourth week of supplementation, the animals of CRE group had renal corpuscles with outlines of

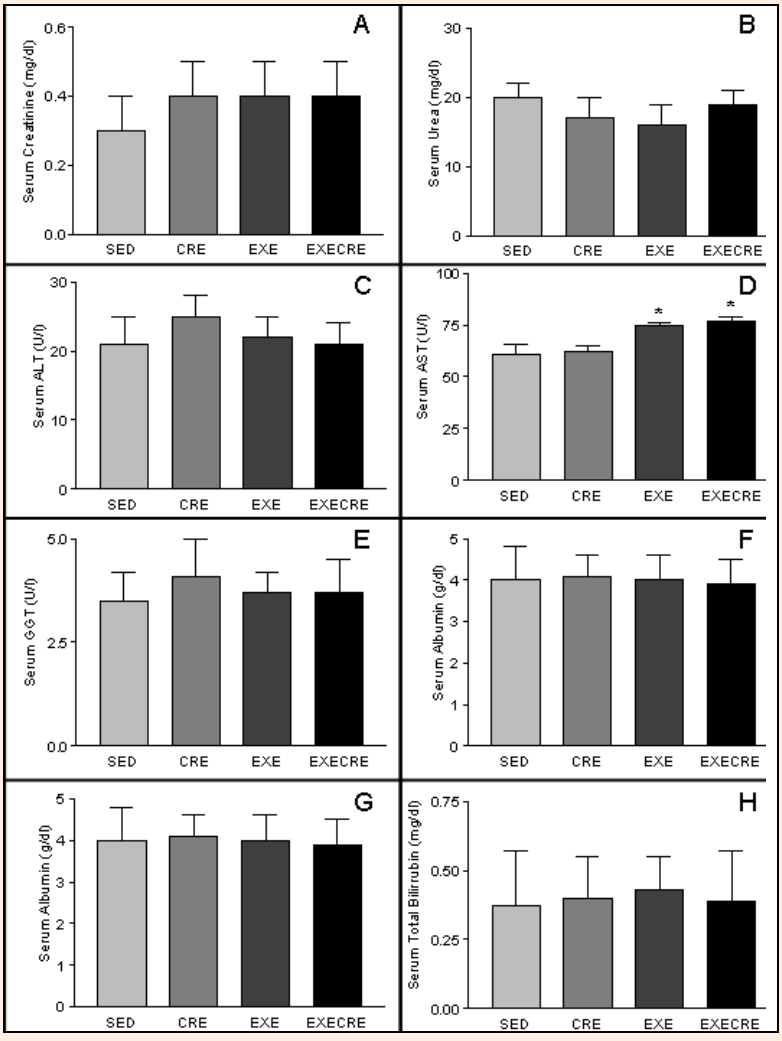

Figure 1. Plasma levels of renal and hepatic markers after 1 week creatine supplementation. Experimental Groups: Normal diet Sedentary (SED), Creatine diet Sedentary (CRESED), Normal diet Exercised (EXE), Creatine diet Exercised (CREEXE). Panels A and B (renal markers): creatinine and urea, respectively; panels C,D,E,F,G and H (hepatic markers): alanine aminetransferase (ALT), aspartate aminetransferase (AST), gamma glutamyltransferase (GGT), alkaline phosphatase (AP), albumin and total bilirrubin, respectively. * $p < 0.05$ when compared with all groups.

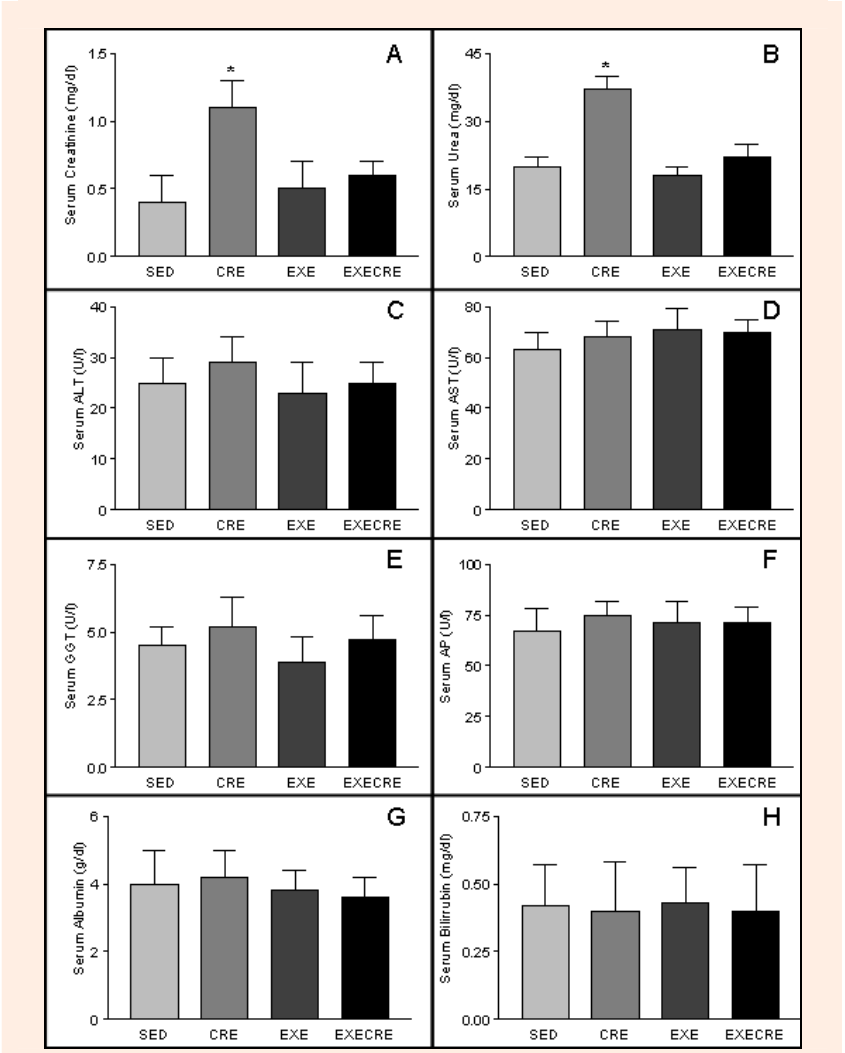

Figure 2. Plasma levels of renal and hepatic markers after 4 weeks creatine supplementation. Experimental Groups: Normal diet Sedentary (SED), Creatine diet Sedentary (CreSED), Normal diet Exercised (EXE), Creatine diet Exercised (CreEXE). Panels A and B (renal markers): creatinine and urea, respectively; panels C,D,E,F,G and H (hepatic markers): alanine aminetransferase (ALT), aspartate aminetransferase (AST), gamma glutamyltransferase (GGT), alkaline phosphatase (AP), albumin and total bilirrubin, respectively. * $p < 0.05$ when compared with all groups.

difficult delimitation and irregular Bowman´s capsules, glomeruli dilations, and no preserved intra-capsular spaces. Renal tubules exhibited cellular morphologic regularity (cuboid epithelium), but indefinite tubular space (Figure 4).

Four weeks of creatine supplementation resulted in no changes of hepatic markers (ALT, AST, GGT, and AP) when compared with all other groups (Figure 2C, D, E and F, respectively; $p > 0.05$), also no changes were observed in liver morphology. However, after 8 weeks of creatine supplementation, the values of ALT, AST, GGT, and AP were significantly higher in the CRE group when compared with the values of all others experimental groups ($p < 0.05$) (Figure 3C, D, E and F, respectively). These functional alterations were accompanied by some histological alterations, like congestion of the central vein, some polymorphonuclear inflammatory cells infiltrate, sinusoids walls showing numerous Kupffer cells with swelling lumen by the blood cells (Figure 5). In contrast, albumin and total bilirubin levels did not present significant alterations during the entire experiment ($p > 0.05$).

Discussion

The key findings from the present study was the demonstration, for the first time, that (a) four weeks of supraphysiological doses of Cr supplementation resulted in increased creatinine and urea levels and renal tissue damage in non trained rats and (b) eight weeks of supraphysiological doses of Cr supplementation produced increased levels of AST, ALT, GGT and AP and hepatic tissue damage in non trained rats. These results suggest that side effects of supraphysiological doses of Cr supplementation on the kidney and liver are time dependent. This study also demonstrated that these possible renal and hepatic side effects are not present in Cr supplemented groups submitted to swimming training (EXECRE), suggesting that the exercise could to block the side effects of high-dose Cr supplementation.

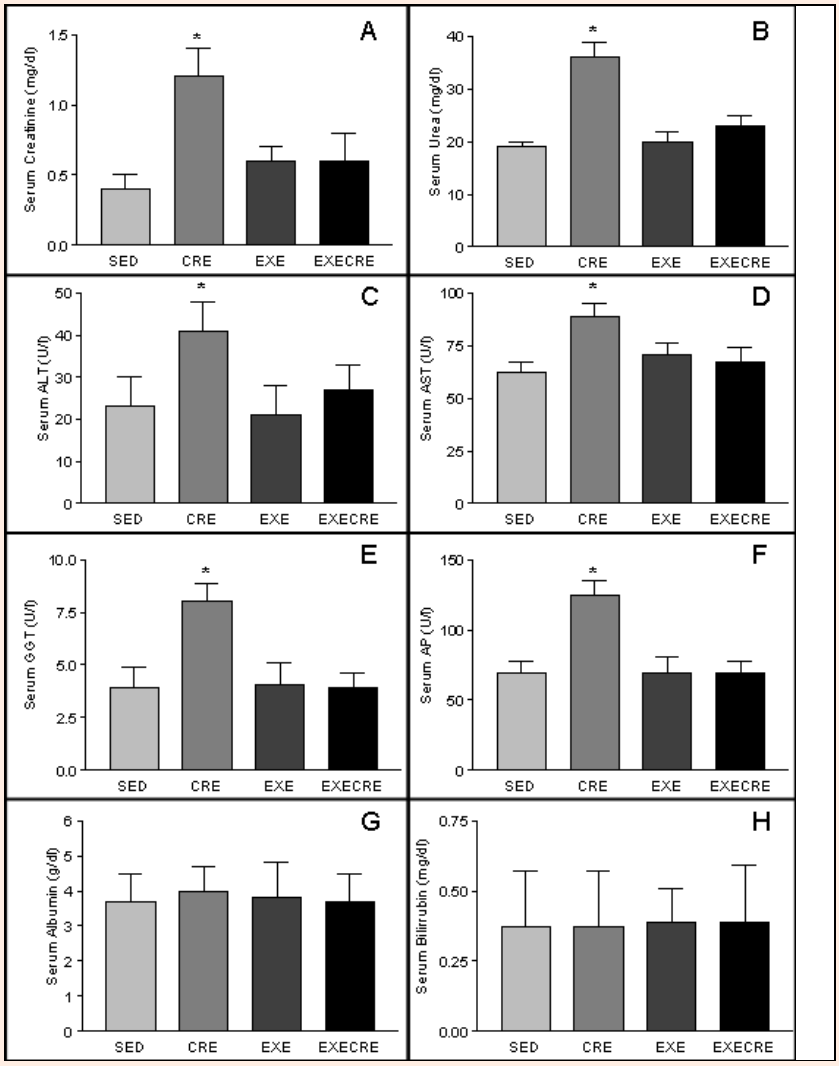

Figure 3. Plasma levels of renal and hepatic markers after 8 weeks creatine supplementation. Experimental Groups: Normal diet Sedentary (SED), Creatine diet Sedentary (CRESED), Normal diet Exercised (EXE), Creatine diet Exercised (CREEXE). Panels A and B (renal markers): creatinine and urea, respectively; panels C,D,E,F,G and H (hepatic markers): alanine aminetransferase (ALT), aspartate aminetransferase (AST), gamma glutamyltransferase (GGT), alkaline phosphatase (AP), albumin and total bilirrubin, respectively. * $p < 0.05$ when compared with all groups.

Oral Cr supplementation in humans and rats has been proven to increase physical performance (Bemben and Lamont, 2005; Kreider, 2003) and some studies have demonstrated benefits in certain pathological conditions (Bender et al., 2005; Jeong et al., 2000). So, the Cr supplementation has become popular among athletes due to a performance-enhancing potential. However, as an available and legal product, it can be easily purchased in nutrition stores or supermarkets, and the potential effect on muscle mass enhancement among untrained and/or among non-healthy people may represent a major concern, because its side effects have not been well established.

The clinical trials studies conducted with Cr using healthy adults are usually divided in two phases: (a) initial phase with supplementation of high Cr doses (20 to 30 g daily) during 5 to 7 days (Loading Phase), immediately followed by (b) a maintenance phase, with small Cr doses, 1/5 of the initial dose (4 to 6 g daily), for several weeks (Bemben and Lamont, 2005; Bird, 2003; Shao and Hathcock, 2006). Using this Cr supplementation protocol, the literature reviews have maintained that there is no conclusive evidence to support the notion that both short- and long-term Cr use may adversely affect kidney and liver function in healthy individuals (Farquhar and Zambraski, 2002; Groeneveld et al., 2005; Kreider et al., 2003; Mayhew et al., 2002; Mihic et al., 2000; Pline and Smith, 2005; Poortmans and Francaux, 1999; 2000; Poortmans et al., 1997; Robinson et al., 2000; Terjung et al., 2000; Waldron et al., 2002; Yoshizumi and Tsourounis, 2004). With respect to the potential side effects of Cr supplementation, kidney damage was based on anecdotal human case reports only when associated with use of high dose Cr or renal disease (Ghosh and Sil, 2007; Koshy et al., 1999; Pritchard and Kalra, 1998; Thorsteinsdottir, et al. 2006).

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 necessary to facilitate demonstration its potential for side effects on

organs that are involved in its metabolism. The choice of the adopted regimen for the Cr supplementation dosage in this study was considered supraphysiological when compared with other animal studies (Brannon et al., 1997; Gagnon et al., 2002; McGuire et al., 2002; Young and Young, 2007).

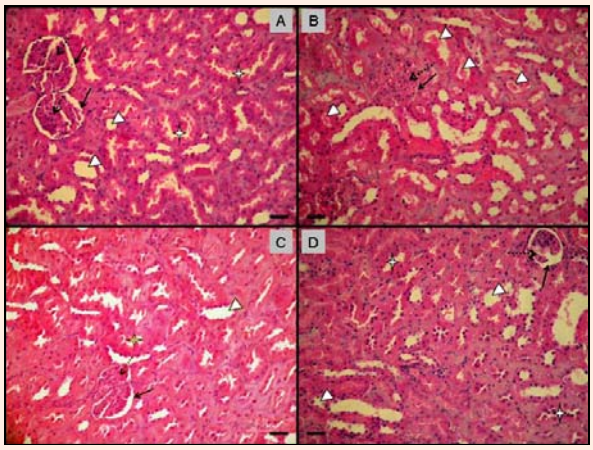

Figure 4. Representative photomicrographs of kidney from Normal diet Sedentary (SED), Creatine diet Sedentary (CRESED), Normal diet Exercised (EXE), Creatine diet Exercised (CREEXE) groups after 4 weeks of supplementation. Magnification 400x. Scale bar = 25µm. The SED group (panel A) had renal corpuscles with a thin contour and well defined Bowman capsules (continuous arrows). Glomerular capilar (dotted arrow) were not congested and had intra-capsular spaces preserved. Renal tubules showed preserved cellular morphology (cuboid epithelium) and distinct lumen, plus proximal tubule convoluted (stars). In CRESED group (panel B), possible renal damage morphology was verified: renal corpuscles with outline of difficult delimitation and irregularity of the Bowman´s Capsule (continuous arrow), dilation of capillary glomeruli (dotted arrow) and intra-capsular space not preserved. Renal Tubules exhibited cellular morphologic regularity (cuboid epithelium), but indefinite space tubular (arrowsheads). Panels A, C and D, show kidney (cortex part) with preserved renal morphology: Renal corpuscles with very defined outline and regularity of the Bowman´s Capsule (continuous arrow), no dilation of glomeruli capillary (dotted arrow) and intra-capsular space preserved. Renal Tubules exhibited cellular morphologic regularity (cuboid epithelium), and definite tubular space (arrowheads).

In the present study we demonstrated that in non trained rats Cr supplementation (CRE group) increased the levels of urea and creatinine, both markers of renal toxicity, at four and eight weeks (Figure 2A-B and 3A-B, respectively). Histological analysis of kidneys of CRE group showed: cortical area with the presence of indefinite renal corpuscles and difficult delimitation of the Bowman´s capsule, congestion of glomeruli capillary, and intra-capsular and tubular spaces not preserved, which indicated tissue damage (Figure 4B). In addition, the same experimental group (CRE) also demonstrated after eight weeks of Cr supplementation increase of AST, ALT, GGT and AP plasma levels of (Figures 3C-F, respectively), and possible hepatic damage morphology was verified: congested central vein with some polymorphonuclear in the peripheral position, walls of the sinusoids showed numerous Kupffer cells and with swelling lumen by the blood cells (Figure 5B). These findings that supraphysiological doses of creatine may negatively impact hepatic and renal function are evidence that the lowest recommended doses of creatine should be followed for the most efficacious and safe outcomes. It is important to explicit that more is not always better (Schilling et al., 2001).

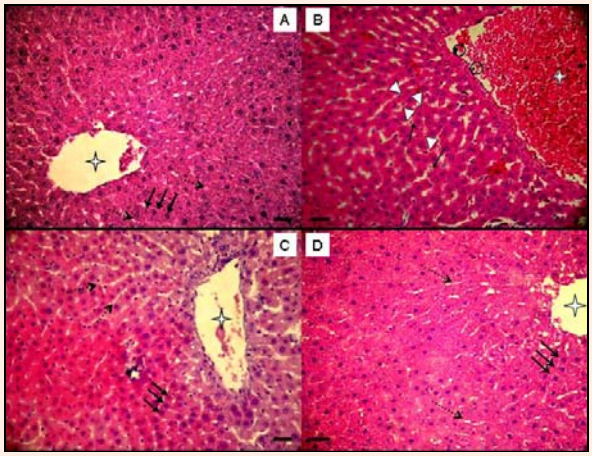

Figure 5. Representative photomicrographs of liver from Normal diet Sedentary (SED), Creatine diet Sedentary (CRESED), Normal diet Exercised (EXE), Creatine diet Exercised (CREEXE) groups after 8 weeks of supplementation. Magnification 400x. Scale bar = 25µm. The SED group (panel A) had a central vein which was uncongested (star), normal hepatocytes (continuous arrows), and slight capillary sinusoids (dotted arrows). The hepatocytes show standard morphology with a big and centralized nucleus. In CRE group (panel B) possible hepatic damage morphology was verified: congested central vein (star) and with some polymorphonuclear in the peripheral position (circular delimitations), walls of the sinusoids showed numerous Kupffer cells (arrowheads) and with swelling lumen by the blood cells (star). The EXE group (panel C) showed hepatocytes (continuous arrows) distributed throughout interconnected plates from the central vein (star) and separated by slight capillary sinusoids (dotted arrows). The EXECRE group (panel D) had preserved hepatic morphology: central vein with no congested (star), hepatocytes (dotted arrows) correctly arranged in trabecules running radiantly from the central vein and separated by thin sinusoids (continuous arrows). They were regular and contained a large spheroid nucleus.

As part of possible toxic mechanisms of Cr supplementation, we could consider the Cr accumulation into the tissue, which has low metabolic capacity to convert Cr into creatinine and is enzymatically capable of accomplishing the metilation processes, contributing to the formation of citotoxic substances, such as formaldeyde and methylamine (Clayton et al., 2004; Yu and Deng, 2000). Additionally, long-term Cr supplementation stimulates down- regulation of Cr receptors (CT-1) in skeletal muscles, blocking any additional storage of this nutrient (Guerrero-Ontiveros and Wallimann, 1998). Greenhaff (1997) demonstrated that muscular Cr capitation is independently saturated after the loading phase, with or without exercise. Therefore, long-term Cr supplementation results in increased Cr concentrations in other organs that present very low basal Cr storage, such as kidneys and liver (Ipsiroglu et al., 2001), which favor the conversion to citotoxic compounds. In the present study, the sedentary animals may have reached their maximum capacity of intramuscular Cr storage, and excessive Cr may have been capitates by renal and hepatic tissue causing some lesion. These findings could justify the elevation of plasma levels of tissue enzymes enzymatic observed in

the CRE group.

It is important also to note that some enzymatic results could also represent a false-positive renal and hepatic toxicity. Since Cr is a substrate for creatinine synthesis, the elevations of creatinine levels observed in the CRE group at four and eight weeks of supplementation could represent simply the result of high-dose supplementation (Poortmans et al., 1997, Yoshizumi and Tsourounis, 2004). Contrary, a recent study does not indicate creatinine elevation after Cr supplementation (Poortmans et al., 2005). Focusing on the endogenous synthesis of Cr, the arginine, one of the precursory synthesized amino acids, also served as precursor for urea production. The Cr supplementation could supply an additional source of arginine to increase urea production (Deshmukh et al., 1991; Ööpik et al., 1996). In addition, in exercised (EXE) and exercised plus Cr supplemented group (EXECRE) after 1 week, we observed an increased level of AST when compared to the Control groups (Figure 1C). These results could be attributed to muscular alterations and not for Cr supplementation, because other studies demonstrate that physical exercise in initial periods of adaptation can generate muscular damage, characterized by plasmatic AST elevation (Peake et al., 2005; Van Der Meulen et al., 1991). However, our results were not attributed to false-positive because use high-dose Cr supplementation affected only the CRE group and it were reinforced by histological findings.

Although many studies on creatine using animal models have been published, few have specifically focused on safety and toxicity and there is evidence that Cr supplementation at doses analogous than those used in humans do not cause adverse effects in most animals under normal conditions (Shao and Hathcock, 2006). For example, Taes et al. (2003) have indicated no deleterious effect in sedentary and in pre-existing renal failure rats. In contrast, Edmunds et al. (2001) and Ferreira et al. (2005) demonstrated creatine-induced deleterious effects in rats with cystic kidney disease and in sedentary animals, respectively. Tarnopolsky et al. (2003) showed significant hepatic inflammatory lesions in mice associated with Cr supplementation, and no negative effect of Cr on liver histology in the Sprague-Dawley rats after intermediate-term or long-term supplementation. These conflicting data may be related to differences in the protocol, species, and tissues analyzed.

In recent years, it has been investigated the reduction of possible side effects of Cr supplementation when associated to physical exercise (Ferreira et al., 2005; Vieira et al., 2008b). Interestingly, although we have used a supraphysiological doses, the renal and hepatic damage were not observed in animals that were Cr supplemented and submitted to swimming training (EXECRE). Researchers suggest that Cr and PCr diffuse between mitochondrial production sites and muscle utilisation sites (Greenhaff, 1997; Walker, 1979). This diffusion process was named as the 'phosphorylcreatine shuttle' and involves 3 areas: (i) a peripheral terminus located at the utilisation site, which in the case of muscle is the myosin heads; (ii) an energy generating terminus which is located at the mitochondria; and (iii) a transversible space between the areas of production and utilisation (Volek and Kraemer, 1996). Although we have no measured the Cr intramuscular it is possible speculate that exercise training could protect against kidney and liver alterations, by increasing Cr consumption by skeletal muscles during swimming, resulting in a decreased Cr accumulation in the kidneys and liver. These results corroborate previous studies which demonstrated that "recommended" doses of Cr supplementation in healthy and active animals did not change the organs function (Ferreira et al., 2005; Vieira et al., 2008b).

Overall, the results of the current study showed that instituting swimming at 80% of the maximal load, five times a week with training daily sessions of 60 minutes may result in a significantly lower side effect of high-dose Cr supplemented for the animal model examined. However, it should be noted that the findings of the current study are not applicable to a Cr supplementation at "recommended" doses.

Conclusion

The results of the current study add to the growing body of knowledge regarding acute and chronic effects of Cr supplementation on renal and hepatic function using an animal model. Therefore, we conclude that physical activity can avoid the development of kidney and liver side effects of long-term supraphysiological doses of Cr supplementation. However, further studies are necessary to clarify the metabolism of long-term Cr supplementation, as well as the possible side effects in organs such as kidneys and liver, and their health consequences, especially in sedentary populations that could to necessity to use this compound.

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Key points

- Creatine supplementation is an established ergogenic aid in sports and is now claimed to have therapeutical applications in a variety of diseases.
- Although acknowledged, this nutritional supplement is rarely monitored precisely about their possible side effects.
- Previous studies indicated that short-term creatine supplementation associate with the physical exercise may be safe, but the effect of long-term creatine supplementation is still unknown.
- There is a need for further research to elucidate the controversial points refers to renal and hepatic function after creatine supplementation.
- The results of the current study indicate that supraphysiological long-term creatine supplementation (up to 4-8 weeks) may adversely affect kidney and liver structure and function of sedentary but not of exercised rats.

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