Insulin resistance with creatine supplementation in laboratory animals*

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ABSTRACT

Introduction and objective: Creatine supplementation has been used in order to improve muscular performance. This substance affects glucose metabolism and stimulates the *in vitro* as well as the *in vivo* insulin secretion. Nevertheless, long-term insulin hypersecretion may also induce insulin resistance. The present work analyzed the effects of creatine oral supplementation in order to evaluate the possibility of occurrence of resistance to *in vivo* insulin. **Methods:** Forty-eight Wistar rats (24 female/24 male) were divided in two groups of 24 (control and study) and subdivided in six groups of eight. They were fed with standard food during four weeks, having water *ad libitum*. Moreover, the study group received dietetic supplement of creatine (0.4 g creatine for 30 ml of water per rat/day). In the 7th, 14th, 21st and 28th day of the experiment, 12 rats were anesthetized (sodium thiopental 0.15 mL/100 g) after six hour-fasting, being submitted to intravenous insulin tolerance test (0.5 mL of 30% regular human insulin and 70% saline solution). The blood samples were collected from the tail veins of the rats, in the basal, three, six, nine, 12 and 15 minutes after insulin administration times. The glucose measurement was performed through the glucose oxidase method. The study was previously approved by the Research Ethics Committee of CCMB- PUC-SP. **Results:** The mean of the glucose decrease constant (K_{ITT}) was calculated through the formula $0.693/T_{1/2}$. The study group, when compared with the control group, presented insulin resistance at day 21 ($p < 0.0004$) and day 28 ($p < 0.0001$). **Conclusion:** This study shows that extended creatine supplementation may lead to insulin resistance. Besides that, it should be carefully used in individuals with glucose metabolism disturbances.

Keywords: Glucose; Dietetic supplements; Sports.

INTRODUCTION

Oral creatine supplementation is generalized in professional as well as amateur athletes in several age groups and it is recommended by the American College of Sports Medicine for performance improvement in short-term exercises and maximal power[1-2]. Creatine is a natural nutrient of animal origin, which is found in meat and fish. It is endogenously synthesized in the liver, pancreas and kidneys, from some amino acids (glycine, arginine, methionine). It is an important energy reservoir for muscular contraction, since around 95% of the body creatine is stored in the skeletal muscle under free or phosphorylated forms (as creatine phosphate – PCr). When the energy demands increase, the creatine phosphate supplies the phosphate for the adenosine diphosphate (ADP) with the aim to synthesize adenosine triphosphate (ATP). This kind of reaction rapidly occurs and results in energy for high-intensity and short-duration physical activities. Creatine supplementation has the objective to increase the content of muscular phosphocreatine; although the results concerning its efficiency are controversial yet[3]. Besides increasing muscular creatine storage, the creatine dietetic supplementation may increase creatine phosphate resynthesis[4], although it has not always been observed[5].

Despite being considered licit and safe by the International Olympic Committee, there are certainly some risks with this supplementation[6]. There are papers which show unwanted effects of creatine chronic supplementation[7] leading to hepatic and renal overload. However, it has not been found in another study[8] renal overload in healthy individuals, even if it shows that those with renal dysfunction should not use the supplementation.

Creatine also affects the metabolism of carbohydrates. When intraperitoneally injected, it leads to hypoglycemia[9] and its supplementation in the diet has shown improvement when there is alteration in the glucose tolerance[10]. Since the 70's, studies with animal models as well as 'in vitro' have demonstrated that insulin increases the blood creatine transportation to the skeletal muscle of rats[11-12]. It has also been demonstrated that creatine increases the muscular storages of glycogen[13]. Glycogen resynthesis[14] and increase of the glucose-carrier protein[15] have been observed in the creatine supplementation, altering the regulation of the glucose metabolism[16-17].

Possibly, the mechanism through which creatine affects glucose metabolism is the stimulation of insulin pancreatic secretion, since, although glucose is the greatest stimulator of insulin secretion, it can also be induced by proteins and amino acids. The role of creatine as stimulator of insulin secretion has been demonstrated in in vitro[18-19] as well as in vivo studies, confirming the very same alterations[16-17].

Nevertheless, this long-term insulin hypersecretion may induce also to insulin resistance[20], which has probably not been previously studied in creatine supplementation. The aim of the present work was to analyze the effects of oral creatine supplementation on the metabolism of carbohydrates, especially to evaluate the possibility of occurrence of in vivo insulin-resistance in animal models (rats) while receiving creatine supplementation for four weeks.

METHODS

The tests were conducted in the Physiology Laboratory of the Center of Medical and Biological Sciences (CCMB), PUC of São Paulo, Sorocaba Campus. Forty-eight albino Wistar rats were selected, 24 males and 24 females, from the CCMB bio cemetery. These animals were divided in eight cages of polyvinyl chloride (PVC) filled with sterilized white pine tree sawdust (approximately 65 cm^2 of area for each animal), with six animals of the same sex distributed in each cage. Four cages were from the Control group and four from the Study group; both groups with two cages with female rats and two cages with male rats. Room temperature was of approximately 20°C and air relative humidity (RH) at around 50-

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55% was kept as ideal for the animals. All animals were fed with the same food (Labina, by Purina) in pellets, 30 g/day per animal) as well as with water ad libitum for the Control group and water with solved creatine for the Study group. The Study group received creatine supplementation in the proportion of 0.4 g of creatine for 30 mL of water per rat/day for four weeks. The experiments occurred on the 7th; 14th; 21st and 28th days of the research. On each day of the experiment, six rats from the Control group and six from the Study group were chosen from the cages (three males and three females each). They were all put on six-hour fasting, beginning at seven o'clock in the morning of the experiment day. For the Study group water with creatine was also removed and later replaced by plain water.

Estimation of in vivo insulin action using insulin-tolerance test of 15 minutes (ITT): At 1 p.m. of the experiment day the insulin-tolerance test began, being the rats intraperitoneally anesthetized (Thiopental Sodium 0.15 mL/100 g), using disposable syringe of 1 mL and 27.5 G ½ needle. Whenever necessary, anesthesia was completed with ethyl ether. A small section in the distal extremity of the rats' tail was performed for blood samples collection. The first collection was conducted prior to the insulin administration (basal). The drops were pipetted with 20 µl pipettes and disposable pipette tips. The sample was placed in ependorff tubes with 100 µl of 5% acid trichloroacetic acid solution (TCA) slightly shaken and stored in a container with ice. Such procedure was identically performed for all groups. Later, 0.5 mL of a 30% regular insulin (Biohulin U-100) and 70% saline solution was administered to both groups in a 1 mL (100 U) sterile and disposable syringe, BD Plastipak brandname, after animal's laparotomy, with the aim to administer the solution in the inferior cava vein. This solution was always kept cold. The blood samples were collected from both groups in the basal (0 minute); three; six; nine; 12 and 15 minute's times, after insulin administration.

All the samples obtained were centrifuged in a centrifuge refrigerated at –4°C, at 3000 rpm for 10 minutes in the Medical Biology Sector; Microbacteria Section, Regional Sorocaba Laboratory of the Institute Adolfo Lutz. The supernatant was pipetted and the glucose measurements were later performed. The remaining of the supernatant was frozen in a conventional freezer at –20°C. At the end of each day, in the four days of the experiment, the animals were sacrificed by the section of the diaphragm muscle.

Blood glucose concentration: The enzymatic method was used for measurement of the blood glucose concentration, using a glucose oxidase kit (Laborlab brandname).

Bioethics: The work was approved by the Ethics in Research Committee of the CMBB-PUCSP according to the specific resolutions for experiments with animals on September 29, 2003.

Statistical analysis: Variance Analysis for repeated values was used[21], with the aim to separately compare for each group the values observed in the basal; three; six; nine; 12 and 15 minutes' times in each of the experiments. T-Student test was used for the comparison of the Study and Control groups concerning the observed values in each of the times mentioned above. One-way Variance Analysis was applied with the purpose to compare the four days for each group in each of the considered times[21].

The ratio of the decrease constant (angular coefficient) of glucose (K_{ITT}) was calculated through the formula $0.693/(T_{1/2})$, being $T_{1/2}$ the time necessary to reduce the basal glycemia in half. The $T_{1/2}$ of the plasma glucose was calculated from the inclination of the decrease curve during its linear phase[22].

The p value was established at 0.05 or 5% the significance level.

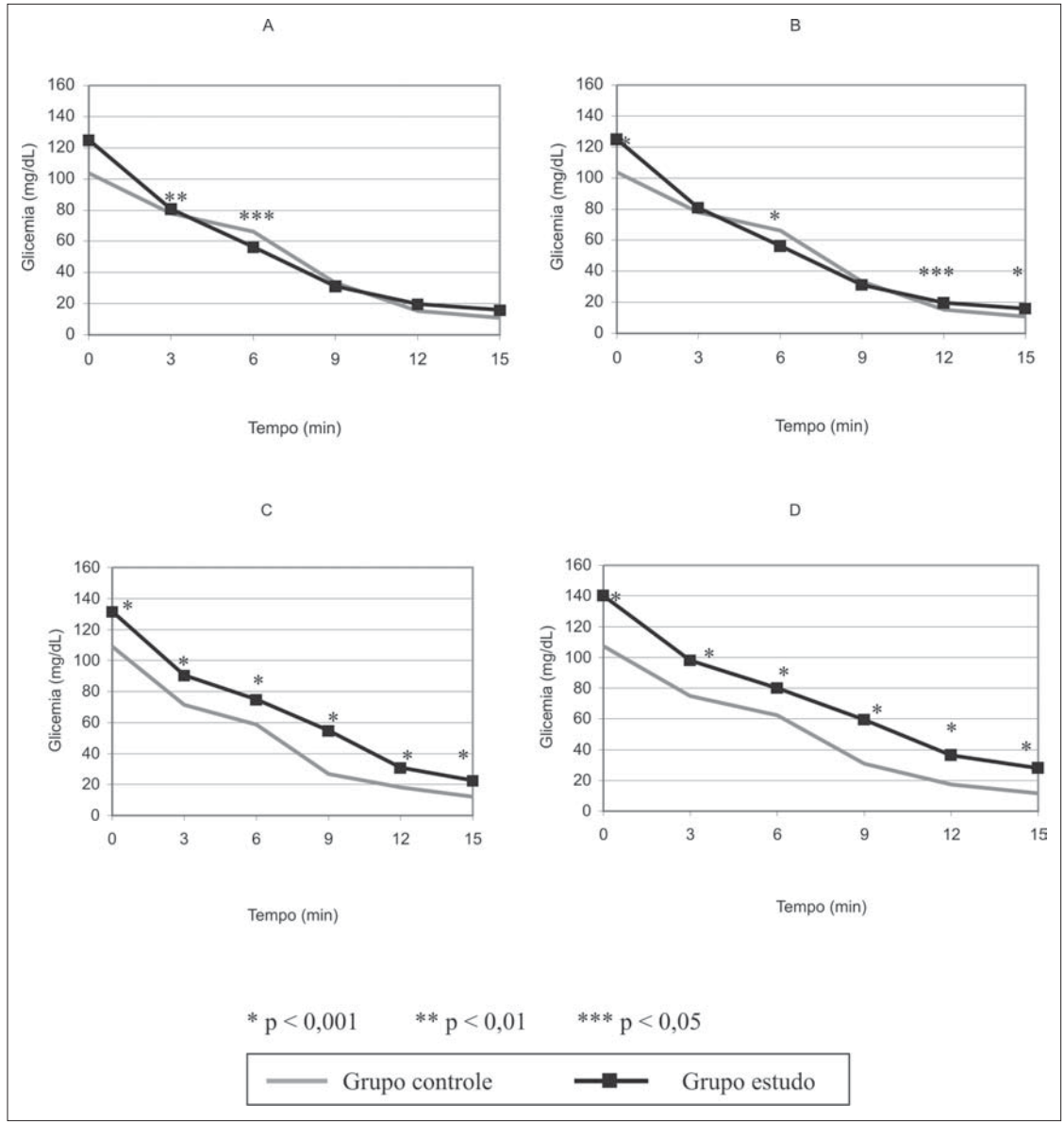


Figura 1 *– Curvas glicêmicas médias nos dias de experimento 7* ***(A)****, 14* ***(B)****, 21* ***(C)*** *e 28* ***(D)*** *nos tempos estudados nos grupos controle e estudo*

RESULTS

The variance analysis showed a significant decrease of the glycemia mean values during the time for both groups ($p < 0.001$) at days seven; 14; 21 and 28. For day 7, the t-Student test showed significant difference between the glycemia means in the three ($p < 0.01$) and 6 ($p < 0.05$) times, with higher values for the Control group. For day 14, the t-Student test showed significant difference between the glycemia means in the basal ($p < 0.001$); six; ($p < 0.001$); 12 ($p < 0.05$) and 15 ($p < 0.001$) times; with higher value for the Control group in time six, and higher values for the Study group in the basal, 12 and 15 times. For day 21, the t-Student test showed significant difference between the glycemia means in all times ($p < 0.001$); with higher values for the Study group in all times. The same situation occurred on day 28: significant difference was shown between the glycemia means ($p < 0.001$), with higher values for the Study group in all times. These data can be seen in figure 1.

The variance analysis for each of the times and days of the experiment showed that in the Control group significant difference was not observed between the mean values of glycemia in each time on the different days. For the Study group, the analysis revealed that in all times the mean values after 28 days were significantly higher than the mean values after days seven and fourteen, and equivalent to the mean values after 21 days ($p < 0.001$). Likewise, the mean values after 21 days were significantly higher than the mean values after seven and 14 days ($p < 0.001$). The values after 14 days were equivalent to the ones after seven days. These data can be seen in figure 2.

The K_{ITT} according to what was mentioned by Bonora *et al.*[22] was used for the estimation of the glucose decrease constant in both groups in the 28 days of experiment. Figure 3 shows that there was significant decrease of the glucose concentration in the Control group when compared with the Study group at day 21 ($p < 0.0004$) as well as at day 28 ($p < 0.0001$), showing that insulin-resistance occurred in the two last weeks in the group which received creatine supplementation when compared with the Control group.

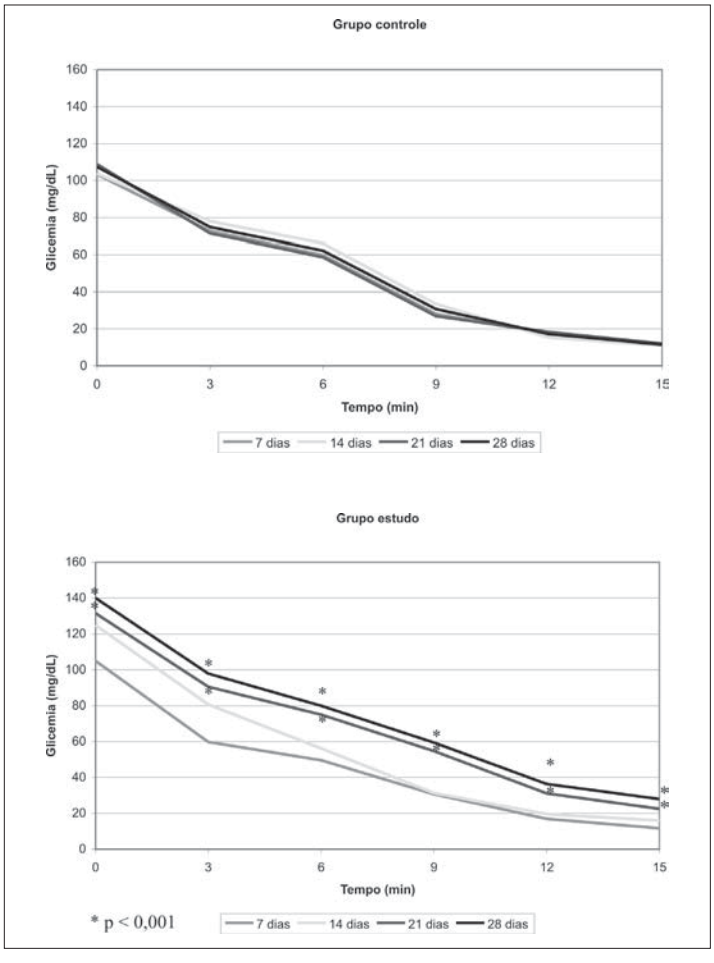
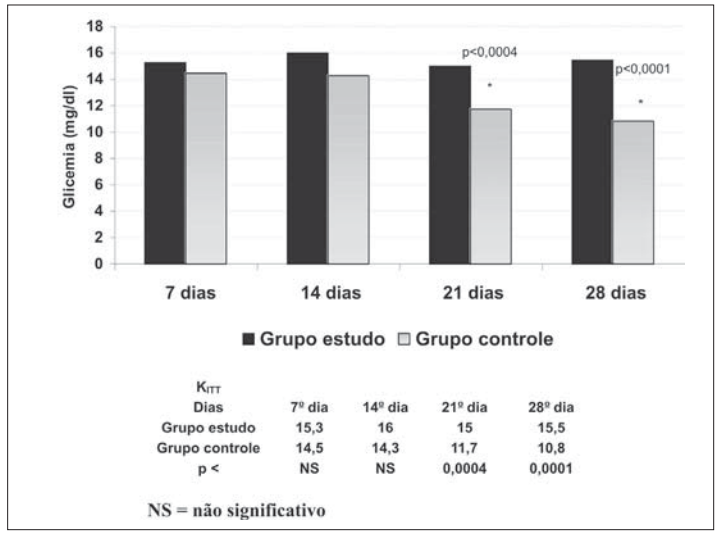


Figura 2 *– Evolução da curva glicêmica média nos grupos controle e estudo*



K_{ITT} Dias	7º dia	14º dia	21º dia	28º dia
Grupo estudo	15,3	16	15	15,5
Grupo controle	14,5	14,3	11,7	10,8
p <	NS	NS	0,0004	0,0001

Figura 3 *– Decaimento da glicose em ambos os grupos nos dias de experimento (K_{ITT})*

DISCUSSION

The creatine administration affects the glucose homeostasis as well as the insulin levels. It has been demonstrated in vitro[18-19] that creatine affects the metabolism of the carbohydrates when directly stimulates the insulin secretion of isolated pancreatic islets. Such fact was confirmed in vivo[16] in a work studying the creatine supplementation in rats in order to observe the long-term effects in the glucose transportation and storage in the skeletal muscle. Moreover, it showed high insulin secretion and alteration in the glucose homeostasis after eight weeks of supplementation when compared with the controls. Thus, it has been demonstrated that there is a relation between the effects of the extended creatine supplementation and its action in the glucose metabolism, with increase of the insulin pancreatic secretion concomitant with a hyperglycemia state. Insulin hypersecretion was not observed with the use of 5 g of creatine[23] or after three days of creatine supplementation in humans[24]; however, these studies do not answer the question of the chronic use of creatine, since the supplementation for a period longer than three days is usual.

Although it has been demonstrated in vivo[16] that chronic supplementation of creatine leads to hypersecretion of insulin, the fact is that the long-term hypersecretion of insulin may also induce insulin-resistance[20], which is the topic of the present study. Having this hypothesis as starting point, we investigated the effects of this hypersecretion of insulin facing chronic supplementation of creatine, searching for evidences of insulin-resistance in rats weekly followed during seven, 14, 21 and 28 days.

The findings of this study demonstrated that creatine supplementary diet led to an increase of the glycemia of the Study group comparing with the Control group. It was seen that after seven days of supplementation there was no significant difference in the glycemic curve between both groups; however, after 14 days of experiment, an increase of the glycemia in the basal and 15 minutes' times was observed in the animals of the Study group. From day 21, it was observed that such increase started to occur for all times, showing that in the Study group the oral creatine supplementation altered the glycemic curve.

As previously described, the K_{ITT} was used in order to confirm the glucose decrease in the 28 days of experiment in both groups[22]. Moreover, it was observed that in the Study group the glucose decrease does not occur as in the Control group, showing that insulin-resistance occurred in animals which received creatine from the last two weeks on.

In a diabetes experimental model, it has been observed that creatine supplementation can improve the sensibility to insulin in extrapancreatic sites[25]. Other works which study the relationship between creatine use and glucose metabolism show that such supplementation, besides the expected benefits in the application in sports medicine, could have other applications. Long-term creatine supplementation improves insulin pancreatic secretion as well as glucose tolerance, it could therefore be applied in patients with diabetes type 2[16]. Although there is no clear definition of the creatine role in the treatment of this disease, two studies comparing the creatine use with medication traditionally used in diabetes – sulphonylurea and metformin – observed that the use of creatine has an effect similar to sulphonylurea in the glycemic control of patients with diabetes type 2[26]; moreover, the use of creatine may be similar to the treatment with metformin in these patients[27].

Conversely, this present study showed that the oral creatine supplementation in rats led to the development of insulin-resistance derived from chronic use of creatine, which causes insulin hypersecretion which would be responsible for the insulin-resistance itself[20]. Although further experimental studies should be developed in order to clarify whether the found metabolic alterations are permanent, as well as studies in humans should be developed for the confirmation of these observations, the hypothesis that creatine supplementation could be benefic for the prevention or treatment of diabetes type 2 becomes incorrect if development of insulin-resistance occurs.

Based on these experimental findings, one may suppose that there may be the development of insulin-resistance in humans in response to its hypersecretion, stimulated by extended creatine supplementation. Professional athletes frequently make use of oral creatine supplementation and, besides suitable physical fitness; they are under medical supervision and monitoring. Nonetheless, it is important to highlight that amateur athletes also use creatine for the improvement of their muscular performance, perhaps with no reliable information sources[28] neither the required medical follow-up, though. Although no important alterations in the metabolism of carbohydrates with short-term use of creatine occurs[29], the information that chronic use of this protein may lead to insulin-resistance should guide evaluation medical protocols of sports activities practitioners, either amateur and/or professional. Such procedure would better control and suitably indicate supplementation, especially of those with previous alterations of glucose metabolism, which would make the use of creatine a considerable risk.

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