Effects of creatine supplementation and power training on performance and lean body mass of rats

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ABSTRACT

Introduction: Creatine is one of the supplements most used by athletes in order to increase protein synthesis and consequently muscle mass and strength. **Objective:** This study investigated the effects of creatine intake on the performance and lean body mass of Wistar rats. **Methods:** Male Wistar rats were allocated into one of the four groups: sedentary without creatine (S); Sedentary with creatine (SC); exercise without creatine (E); and exercise with creatine (EC) and received water and chow *ad libitum*. Those animals in SC and EC groups ingested creatine daily (0.430 g/kg body weight for 7 days and 0.070 g/kg body weight for the following 6 weeks). Animals from E and EC groups underwent a progressive vertical jump regimen (5 x 10 jumps with 1 min. resting interval) in a tank filled with water at 30 ± 1ºC, 5 days/wk for 7 weeks. Performance was assessed by taking the time to perform 5 x 10 vertical jumps. The contents of water, fat and protein of the rat's muscles and bones were measured. **Results:** The performance was not affected by creatine intake ($P > 0.05$). Animals supplemented with creatine had an increased percentage of protein and a reduced percentage of fat ($P < 0.05$), regardless the exercise training. Exercised animals exhibited a higher percentage of protein and a lower percentage of fat and gained less body weight when compared to sedentary animals ($P < 0.05$), regardless the creatine supplementation. There was no difference between groups for water content and food intake ($P > 0.05$). **Conclusion:** Creatine supplementation did not affect performance of the animals. Nevertheless, it altered the lean body mass. Creatine supplementation as well as the power training program, independently, raised the protein percentage of the muscles and bones and reduced the fat percentage, with no alteration in the water percentage.

Keywords: Physical activity. protein synthesis. Retention of water.

INTRODUCTION

Creatine is found in the skeletal muscle (~95%), as free creatine (Cr, ~40%) and phosphocreatine (PCr, ~60%)[1-2]. Its absorption in the intestinal tract is by active transportation, with its largest part of ingested doses removed from plasma by the kidneys and excreted in the urine[3]. The creatine daily turnover rate in normal individuals is 2 g/day, derived from exogenous sources or endogenous synthesis[4-6]. The main functions of creatine are related with the supply of temporary energy, the energy transportation between the production and consumption sites and the maintenance of the ATP/ADP resynthesis rate[5,7]. Creatine also promotes the supply of hydrogen protons and regulates glycolysis[4,7]. The PCr degradation through an irreversible reaction generates creatine as a final product[8], which is excreted in the urine[5]. Thus, creatine supplementation induces to greater urinary creatine excretion, possibly due to the increment of creatine body supplies[1,3,9].

The use of creatine supplementation as an ergogenic aid has increased among athletes of modalities in which muscular mass and power are decisive for performance[1-2,5], especially in activities of short duration and high intensity[2-3,10-11]. Its benefits for performance during physical exercise are associated with increase of the intracellular energy, increase of the PCr resynthesis rate, reduction of the inorganic phosphate accumulation and increase of pH[5,12-13]. There is evidence that performance in repeated sprint bouts[6,14-15] and power exercises[10,13] increased after creatine intake, which promotes the ATP resynthesis and increases the PCr availability and degradation[8,13,16]. However, such evidence has not been confirmed by other studies using short duration and high intensity exercises[17-19]. Such controversial findings may have occurred due to different used methodologies, for instance: creatine dose, kind of exercise and kind of supplementation.

The increases of muscular mass and strength are crucial for performance in the majority of power exercises. Studies show that creatine supplementation can increase body weight and strength in athletes[2,4,18]. Some researchers argue that the increase of body weight in response to strength exercise practice and creatine supplementation occurs due to greater water retention in muscles caused by the osmotic effect derived from the increase of intramuscular creatine[11,15,20-21], despite some evidence which proves the contrary[22]. Another explanation is the reduction of the degradation and increase of protein synthesis. It is argued that the cellular edema derived from the water retention, in response to creatine intake, attenuates the protein degradation rate since it reduces the release of the branched-chain amino acids (BCAA), returning to normal when the cell reestablishes the normal conditions, suggesting that creatine reduces the muscular proteolyse[23]. McClung *et al.*[24] showed that the interaction between creatine intake and physical exercise increased the protein synthesis of the cardiac muscle of rats, but it did not increase the total body protein. The study by Ziegenfuss *et al.*[15] demonstrated that cyclists who cycled in high intensity and short duration (6x10'' with 1' rest) and ingested creatine (0.350 g/kg/day) had increased body weight and muscular volume; these mechanisms have not been explained, though. However, Louis *et al.*[22] demonstrated that the association of creatine acute intake with strength exercise did not show anabolic effect in protein synthesis rate. Moreover, it is speculated that indirectly, creatine supplementation would enable athletes to perform a greater exercise load due to its ergogenic effect[25], which improves the protein synthesis. Therefore, the chronic creatine supplementation effects associated with chronic power exercise over the lean body mass composition are not well-defined yet. The studies with athletes did not make these direct analyses possible.

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Thus, the aim of this study was to investigate the effects of creatine supplementation associated with a power training program (vertical jumps) over the performance and lean body mass composition of Wistar rats. Our hypothesis is that the creatine supplementation associated with vertical jumps training increases performance and protein percentage in the lean body mass.

MATERIALS AND METHODS

Experiment animals and treatment: Thirty adult animals (*Rattus norvegicus* – Wistar) (Weight: 350.0 ± 11.9 g; mean ± SD) were randomly distributed in four groups: S (sedentary without creatine; n = 08); SC (sedentary creatine; n = 08); E (exercise without creatine; n = 06); and EC (exercise creatine; n = 08). The animals were individually placed in stainless steel cages and kept in environment with mean temperature of 24ºC and light regimen of 12 h light/dark. All animals received commercial chow (Socil®) and destilled water *ad libitum*. The animals were provided by the Central Animal Facility of the Biological and Health Sciences of the Federal University of Viçosa. The regulations of care for animal experimentation were followed according to the Brazilian College of Animal Experimentation (COBEA).

Creatine Administration: The SC and EC groups received a daily creatine supplementation, with two phases of procedure: load and maintenance. The load phase occurred during seven days starting on the second week of experiment, with the creatine dose (Power Nutrition®) of 0.430 g/kg of body weight/day added with the same volume of maltodextrine (Neo-nutri®). The maintenance phase lasted seven weeks, starting in the third week, with creatine dose of 0.070 g/kg of body weight/day added with the same volume of maltodextrine. The S and E groups received supplementation only with maltodextrine identical to the other groups. Such doses were determined based on the 30 g dose in the load phase and 5 g in the maintenance phase for a 70 kg man.

Exercise Program: On the first week of experiment, the animals of the E and EC groups were placed in a tile tank (width: 60 cm, length: 75 cm and height: 80 cm) with water (~33ºC) at 15 cm depth, for 30 daily minutes, for adaptation to the water temperature and medium.

The exercise program was performed according to Oliveira *et al.*[26], with the following adaptations: animals were placed inside PVC pipes (diameter: 25 cm and height: 60 cm) closed in their inferior extremity with nylon net. The exercise overload (body weight percentage) was added to the animal using lead spheres inserted in a lycra vest which the animals were wearing. The water depth was determined by a mean of the percentage of the animals length measured by the longest distance between the extremities of the posterior limbs and nostrils. The exercise consisted of vertical impulse jumps from the bottom of the tank (feet touch) until water surface (nostril out of the water). The exercise load progression is showed in table 1.

TABLE 1
Load data of the exercise program

Week	Bouts	Jumps	Load	Water depth
1st			Water adaptation – 30'	80%
2nd	4	10	20-25%	120%
3rd	4	10	30-35%	130%
4th	4	10	40%	140%
5th	4	10	45%	150%
6th to 8th	5	10	50%	150%

Determination Body Weight, Weight Gain and Food Intake: All animals were weekly weighted for determination of body weight gain during the experimentation period. Food intake was daily monitored.

Determination of Urinary Creatine: Urinary creatine was determined to confirm the use of the PCr as energetic substrate used during the intermittent anaerobic exercise performance. Moreover, it enabled the verification of the effects of supplementation and exercise under creatine excretion. For creatine analysis, the animals' urine was collected for a period of 24 hours using stainless steel metabolic cages. The urine volume collected was completed for 10 mL with deionized water and centrifuged for 15 minutes at 4.000 rpm (Excelsa-Fanem, Brazil). From the supernatant of the centrifuged urine, 50 μL were pipeted in cuvet and diluted for 500 μL with deionized water for determination of urinary creatine through automatized method of espectrometry by UV/VIS[27]. The analyses were performed using the kit by Bioclin® in ALIZÉ® equipment (Biomêrieux-France) by the *Biopharmacos* Laboratory of the Federal University of Viçosa.

*Performance Evaluation***:** The exercise performance was evaluated by the time spent to perform each bout of ten vertical jumps. After the training period an exercise bout (5x10 jumps) was performed where the time spent in the performance of ten jumps was registered (digital Casio® Stopwatch HS-30W). The stopwatch was started when the animal lost contact with the ground at the first jump and stopped when the animal reached the water surface with the nostrils at the tenth jump.

Determination of the Lean Body Mass Composition: At the end of week eight, the animals were slaughtered and the skin, viscera, head and feet were discarded. Only the bones and muscles remained (empty carcass) for quantitative analysis of water, fat and protein water according to Pitts *et al.*[28]. In the determination of water content, the empty carcasses were individually placed on aluminum plates and introduced in a drier at 105ºC for 24 hours. The carcass water was calculated by the difference of the pre and post drying carcass weight. After drying, the empty carcasses were macerated and placed in paper filter tubes for fat extraction by the Soxhlet method during eight hours, using petroleum ether as solvent. Fat percentage was determined by the difference of the weight of the tube containing the pre and post degreased carcass. The protein percentage was three times calculated by the indirect method of nitrogen determination by the Kjeldahl method[29], using the 6.25 factor for conversion into protein. The analyses were performed in the laboratories of the Nutrition and Health Department of the Federal University of Viçosa.

Statistical Analysis: The data were evaluated by analysis of variance: two-way ANOVA and t-student test for results among groups and repeated measurements ANOVA for results between weeks of each group. The Tukey test was applied for analysis of *post-hoc* multiple comparison whenever necessary. The software Sigma Stat version 3.0 (SPSS) was used for the statistical analyses, applying the statistical significance level of $P < 0.05$.

RESULTS

Body Weight, Weight Gain and Food Intake: Significant difference was not verified in the initial body weight among groups ($P > 0.05$; table 2). Statistical difference was not observed either in the body weight among groups in the second (S: 367.5 ± 5.5; SC: 366.3 ± 5.5; E: 348.3 ± 6.3; EC: 365.0 ± 5.5 g; Mean ± SD) and third weeks (S: 370.0 ± 6.0; SC: 366.2 ± 6.0; E: 346.3 ± 7.0; EC: 367.0 ± 6.0 g; $P > 0.05$). However, significant difference in the body weight among groups in the eight weeks was observed ($P < 0.05$; table 2), where E was lower than EC and S. Significant increases in body weight were observed between weeks 1 and 8 of experiment in the groups EC, S and SC ($P < 0.05$), which did not occur in the E group ($P > 0.05$; table 2).

The final body weight presented statistical difference due to the exercise and creatine factors. The exercised groups (EC/E) reduced body weight compared with the sedentary ones (SC/S, $P < 0.001$), and the supplemented groups (EC/SC) have raised it comparing to

TABLE 2
Body weight in weeks 1 and 8, weight gain and food intake

	Body weight (g)		Weight gain (g)	Food intake (g)
	Week 1	Week 8		
S	351.3 ± 4.4##	402.5 ± 5.8#	38.8 ± 6.6#	1,365.0 ± 41.7#
SC	350.0 ± 4.4##	395.0 ± 5.8	35.0 ± 6.6	1,326.3 ± 41.7
E	346.7 ± 5.1	343.3 ± 6.7	–05.0 ± 7.7	1,203.3 ± 48.2
EC	351.3 ± 4.4##	376.3 ± 5.8#	22.5 ± 6.6#	1,350.0 ± 41.7#
Exercise				
Sedentary (S + SC)	350.6 ± 3.1	387.5 ± 4.2	36.8 ± 4.7	1,345.6 ± 29.9
Exercised (E + EC)	349.0 ± 3.4	357.7 ± 4.6*	8.8 ± 5.1*	1,276.7 ± 31.9
Supplementation				
Without Creatine (S + E)	349.0 ± 3.4	365.8 ± 4.6	16.9 ± 5.1	1,284.2 ± 31.9
Creatine (SC + EC)	350.6 ± 3.1	379.4 ± 4.3**	28.8 ± 4.7	1,338.1 ± 29.5

Data are means ± SD. Significances (P < 0.05): ## *vs* week 8; E; # *vs* E; * vs Sedentary; ** *vs* Without Creatine.

the one without supplementation (S/E, P = 0.039; table 2). Significant reduction in body weight gain was observed for the exercised groups compared with the sedentary ones (P < 0.001; table 2), as well as a significant interaction between exercise and creatine. The E group lost weight compared with the EC (P = 0.012) and S groups (P < 0.001; table 2).

Statistical difference was not observed in food intake for the exercise and creatine factors during the experiment (P > 0.05; table 2); nevertheless, a statistical interaction was observed between these factors. The E group presented lower food intake than the EC (P = 0.030) and S groups (P = 0.018; table 2). Moreover, body weight gain presented a regular positive correlation with food intake (Pearson Correlation, r = 0.452 and P = 0.012).

Performance in the Jumps: This evaluation was conducted only with the exercised groups. Statistical difference was not observed in the performance time between bouts of each group (Friedman Repeated Measurements, EWC: P = 0.103 and EC: P = 0.112), nor between the E and EC groups in each bout (bout 1: P = 0.687; bout 2: P = 0.108; bout 3: P = 0.122; bout 4: P = 0.228; and bout 5: P = 0.090; figure 1). Statistical difference was not found either for the total time of the five jump bouts between groups (E: 188"1 ± 116"4 *vs* EC: 111"6 ± 25"2 seconds; P = 0.093).

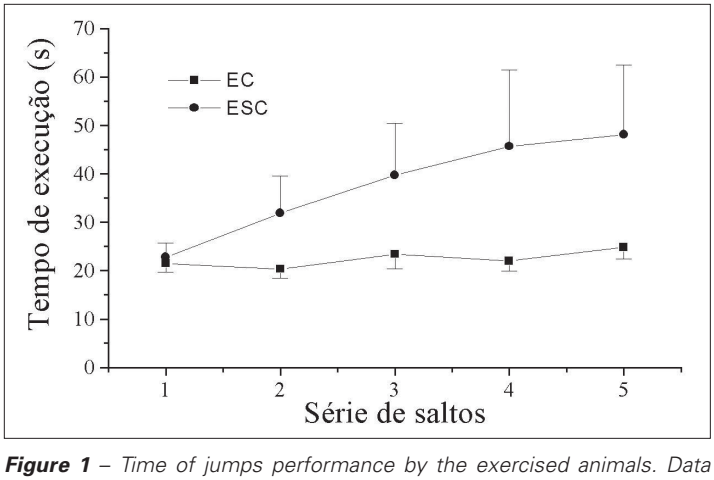

Figure 1 *– Time of jumps performance by the exercised animals. Data presented in mean ± MSE.*

Urinary Creatine: The S and SC groups presented urinary creatine excretion higher than the E and EC groups (P = 0.008 and P < 0.001; respectively; table 3). Creatine supplementation did not significantly alter the urinary creatine excretion or the relative creatine rate, neither interaction with exercise in both parameters was observed (table 3). Significant alteration between groups was identified between groups for creatine relative rate (ANOVA, P > 0.05). However, it has been observed that the exercised groups presented lower creatine excretion and relative creatine rate compared with the sedentary groups (P < 0.001; table 3). There was regular positive correlation of urinary creatine with the final body weight (Pearson Correlation, r = 0.560 and P = 0.001).

TABLE 3
Urinary creatinine and creatinine rate/body weight

	Creatinine (mg/24h)	Creatinine/Weight (mg/24h/g)
S	103.39 ± 9.1	0.256 ± 0.023
SC	128.71 ± 9.1	0.325 ± 0.023
E	63.58 ± 10.5#	0.188 ± 0.027
EC	59.14 ± 9.1##	0.157 ± 0.023
Exercise		
Sedentary (S + SC)	127.93 ± 6.3	0.331 ± 0.017
Exercised (E + EC)	59.88 ± 6.8*	0.169 ± 0.018*
Supplementation		
Without Creatine (S + E)	92.93 ± 6.8	0.251 ± 0.018
Creatine (SC + EC)	94.87 ± 6.3	0.248 ± 0.017

Data are means ± SD. Significances (P < 0.05): # vs S, ## vs SC, * *vs* Sedentary.

Lean Body Mass Composition: The weight of the empty carcass and its water percentage did not present significant difference among groups, nor concerning the creatine and exercise factors (ANOVA, P > 0.005; table 4). The fat percentage was not significant different among groups; however, statistical differences were identified for creatine and exercise factors, the supplemented and exercised groups showed lower fat percentage in the carcass compared with their control groups (P = 0.003 and P = 0.017; respectively, table 4). Concerning the protein percentage, the supplemented and exercised animals showed significant increase compared with their control groups (P = 0.002 and P < 0.001; respectively, table 4). It was also observed that the S group presented lower protein percentage than the SC and E groups (both P < 0.001); and group EC presented higher protein percentage than SC (P = 0.041; table 4).

DISCUSSION

In this study the effects of creatine intake and a training program with vertical jumps over performance and lean body mass composition of rats were investigated. Our results showed that the creatine intake stabilized the performance time of vertical jumps, that the exercise program and creatine supplementation independently promoted increase of protein incorporation and reduction of the fat percentage, but did not affect the water content.

TABLE 4
Composition of empty carcass

	Empty carcass G	Water %	Fat %	Protein %
S	166.8 ± 4.0	69.2 ± 0.3	4.1 ± 0.2	21.4 ± 0.2
SC	165.6 ± 4.0	69.2 ± 0.3	3.2 ± 0.2	22.5 ± 0.2#
E	164.6 ± 4.6	69.7 ± 0.4	3.3 ± 0.3	22.9 ± 0.2#
EC	163.8 ± 4.0	68.9 ± 0.3	2.9 ± 0.2	23.2 ± 0.2##
Exercise				
Sedentary (S + SC)	166.2 ± 2.8	69.2 ± 0.22	3.70 ± 0.18	21.9 ± 0.14
Exercised (E + EC)	164.2 ± 3.0	69.3 ± 0.24	3.08 ± 0.19*	23.0 ± 0.50*
Supplementation				
Without Creatine (S + E)	165.7 ± 3.0	69.5 ± 0.24	3.76 ± 0.19	22.1 ± 0.15
Creatine (SC + EC)	164.7 ± 2.8	69.0 ± 0.24	3.03 ± 0.18**	22.9 ± 0.14**

Data are means ± SD. Significances (P < 0.05): # *vs* S; ## *vs* SC, * vs Sedentary; ** *vs* Without Creatine.

In the present study it was observed that acute creatine supplementation, in the load phase, did not alter the body weight (table 2). In the maintenance phase though, the chronic creatine intake increased the body weight in 3.7%. According to previous studies, the alteration of body weight due to creatine supplementation has shown contradictory effect, since some authors have shown increase[9,30] while others did not observe alterations[22]. The increase in the final body weight observed between the EC and S groups, compared with the E group (9.4 and 14.1%, respectively) may have justified by the increase of food intake in these groups (12.2 and 13.5%, respectively, table 2). Such fact is confirmed by the regular positive correlation observed between body weight gain and food intake. On the other hand, body weight gain is associated with higher water retention derived from the osmotic effect caused by the increase on intramuscular creatine[4,21], which was not observed in this study (table 3), corroborating the hypothesis that higher food intake was responsible for the increase of body weight in the supplemented groups. The great reduction in body weight in the exercised groups was not expected, since the exercise kind and duration developed would not be determinant factors in this reduction observed. Sessions of five bouts of ten intermittent jumps were performed between five and seven minutes a day. This duration time would not promote high energy cost, and consequent body weight loss. However, lower energy cost could occur due to the excess of the EPOC effect promoted by high intensity and short duration exercise[31]. Although the creatine and exercise factors have resulted in significant increases in the final body weight, these differences cannot be attributed to the empty carcass water, protein and fat percentages, since the percentage of protein increased, the fat one decreased, and the water one did not alter for both factors. Moreover, the empty carcass weight did not suffer significant alteration among groups and/or factors. Thus, the explanation for this observed difference may be in the greater accumulation of visceral fat due to higher food intake. Nonetheless, in this study, the viscera, head, tail and skin were discarded.

In the present study, the creatine supplementation did not affect the urinary creatinine excretion. This result differ from other studies in humans, where creatine supplementation induced higher urinary creatinine excretion, possibly due to the increase of body creatine supplies[3,9]. On the other hand, there was reduction of relative urinary creatinine in week eight due to the exercise program, which was not expected. Studies have shown that short duration anaerobic exercise increases the urinary creatinine once it increases the PCr catabolism during its performance[9,13,19,32], since in this kind of exercise the needed energy is provided by the ATP-CP system, resulting in greater production of creatinine. Our results could be explained by a possible increase of anaerobic glycolysis during the exercise performed during the experiment, due to the products of the PCr hydrolysis (Cr and Pi) activate this metabolic way[13]. Another possibility would be the occurrence of a lower muscular catabolism in the exercised animals and, consequently, lower creatinine excretion. Creatinine is used as a marker of muscular mass, once it is the site of highest creatine storage[33-34], and in the present study it was also verified that the protein percentage also increased due to exercise (table 4). Nevertheless, it is possible that magnitude of creatinine reduction in the exercised groups is not explained by these mechanisms, which lead us to suggest that further studies using creatine and creatinine marked with isotopes which may identify the real way of creatine utilization and the origin of the excreted creatinine after exercise should be conducted.

Although our results do not present significant difference for the jump times in the groups, at 5% of probability, it is worth mentioning that at 10% the difference was significant. The total times of the bouts and the time of the fifth jump bout showed that the lack of creatine supplementation increased the time of the jumps when compared with the creatine intake (P = 0.090). In addition to that, the differences of the times and their meanings for performance are expressive. The group without creatine supplementation increased in 40.7% the total time of jumps and in 48.4% the time of the fifth bout, compared with the group which ingested creatine. Creatine intake increases power exercise performance since it increases the PCr availability for the ATP resynthesis, which is the main limitation of high intensity and short duration exercise[3,12-13,19]. Moreover, creatine intake reduces post-fatigue recovery time[13], which leads us to speculate if the performance of a higher number of bouts would evidence the effect of creatine supplementation over performance in the present study. Lemon[25] reports that the power of the high intensity exercise and the muscular mass in humans increased after 36 days from the creatine intake (0.300 g/kg/day). In this investigation, the supplemented animals presented higher body protein percentage which would promote greater contractile capacity for the performance of the alactic anaerobic exercise[9]. These findings, compared with the athletic performance, may represent an expressive difference. Performances in a sequence of jumps in volleyball or in a sprint at the end of a game depend on the greater capacity in performing a high intensity and short duration exercise.

An important result of the present study was the increase in protein percentages in carcasses of exercised animals (5.1%, table 4), regardless supplementation, corroborating the capacity of the exercise model used in developing muscular hypertrophy. Our outcomes were similar to others[26] in Wistar rats whose relative empty carcass composition (muscles and bones) presented the following values: similar water (69.7 ± 0.5%), fat (4.8 ± 3.2%) and protein (21.2 ± 1.8%), using similar analyses method. The absolute values of the body fractions of our study (carcass: 165.2 ± 10.7; water: 114.4 ± 8.1; fat: 5.6 ± 1.3; and protein: 37.1 ± 2.8 g) were similar to the ones shown by Pitts *et al.*[28] (carcass: 185.6 ± 4.2; water: 129.4 ± 4.0; fat: 9.4 ± 1.8 g; and protein: not performed).

Anaerobic training results in hypertrophy of the muscular fiber, reflected in the muscle size, due to the protein liquid balance[9,11]. Hornberger and Farrar[35] showed that vertical climbing (80º of inclination) with overload, weight tied to the tail, during eight weeks, increased the myofibrillar and total protein of rats in 24%. In humans, Louis *et al.*[22] showed that, 20 bouts with 10 repetitions increased from 2 to 3 times the synthesis rate of the myofibrillar and sarcoplasmatic proteins. Our data demonstrate that the jumping exercise model in water used in the present study causes adaptations inherent to the strength training.

In addition to that, it was observed in this investigation that the creatine supplementation for seven weeks increased the animals' muscular protein, regardless the exercise factor (table 4). Lemon[25] suggests that the creatine ingested in a dose of 0.300 g/kg/day during 36 days promotes a gain in muscular mass. Although data of body composition in creatine supplemented rats have not been found, our results were similar to the ones in studies with humans[3,14], showing that the creatine intake (0.333 g/kg/day) for ten and six weeks increased the lean mass, when evaluated by hydrostatic weighting. Mendes *et al.*[19], by electric bioimpendance, demonstrated increase in lean mass of swimmers after one week of creatine intake (0.286 g/kg/day). These outcomes could be justified by the possible creatine direct effect in reducing the oxidation rate of leucine and proteolysis (anti-catabolic action) or increasing the protein synthesis. However, since this study did not observe alteration in the carcass water content, it is not possible to affirm that the increase in the incorporation of body protein would be derived from greater intramuscular water volume, which would alter the nitrogen balance inducing a higher rate of protein synthesis[23].

Despite having observed an increase in the protein percentage of the animals concerning with the creatine and exercise factors, in this study no difference between the EC and E groups was observed, suggesting hence that there is no interaction between these factors in the alteration of protein synthesis. It is worth highlighting that such interaction was observed by McClung *et al.*[24], only in the cardiac muscle.

Based on data from other studies[2,4,21], it was expected that the creatine incorporated to the intramuscular medium increased the osmotic effect retaining greater water content in muscles, and therefore, increasing body weight. Nevertheless, in the present study creatine intake did not alter the water percentage in the empty carcass. These outcomes are contrary to the ones found by Mendes *et al.*[19], who observed increase in the body water content through electric bioimpendance in relation to the creatine intake; however, they suggested the performance of muscular biopsies in order to confirm such results. Volek *et al.*[9] have also observed increase of body water of athletes supplemented with creatine, but did not find difference in the total body water content, expressed in body weight percentage. In the present study the empty carcasses of the animals were analyzed due to the fact that 95% of the ingested creatine is stored in the skeletal muscle as Cr and PCr[1-2]. Therefore, our data do not support the theory that muscular water is retained due to creatine supplementation.

In this study, exercise reduced the final body weight (8.3%) and the fat percentage of the empty carcass (20.1%) after eight weeks of training (tables 2 and 4). These alterations are credited to endurance training[36]. However, it is believed that this reduction did not occur due to the direct energy cost of the exercise, but because the anaerobic exercise promotes an oxygen deficit, increasing the post-exercise energy cost. The oxygen cost remains high after the anaerobic exercise due to active muscular biochemical processes, increasing the energetic metabolism, called 'excess post-exercise oxygen consumption' (EPOC)[31,37].

Lower fat percentage in the carcass due to creatine supplementation (table 4) was not expected, since some studies[3,14,23] show that creatine supplementation does not affect the fat oxidation during exercise and the body fat content in humans. A single report was identified in the study by Volek *et al.*[9], where humans evaluated by DEXA, presented lower total fat content, in the arm, leg and chest due to creatine intake. However, no justification for this finding was presented. This fact suggests the need for further investigations in order to evaluate the possible physiological or metabolic mechanisms for this effect.

Facing what was presented, it was concluded that creatine supplementation did not affect performance of animals, but altered lean body mass. Creatine supplementation and the power training program, independently, increased the protein percentage of muscles and bones and reduced the fat percentage, with no alteration on water percentage.

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