ABSTRACT

Despite scientific advances and findings on the effects of creatine supplementation, little is known about the use of this supplement and its effects on visceral fat accumulation, as well as on the process of improving muscle endurance associated with stereological factors of muscle tissue. This study aimed to evaluate the effects of creatine monohydrate use on physical valences, stereology of muscle tissue and quantitative aspects of visceral adipose tissue. Twenty-four Wistar rats, aged 15 weeks and under controlled feeding and hydration conditions, were selected. To verify the desired variables, the animals were classified into four groups: control, supplemented, training, and training and supplemented. Creatine supplemented animals were supplemented (via gavage) at dosages of 300mg / kg body mass for nine weeks, and the same period of training as the animals that were trained underwent a program based on Honenberg & Farrar studies. The loads at which the animals performed the series were noted, as well as the number of climbs performed per training session. After the experimental period, the animals were weighed and euthanized, and visceral fat was removed for weighing and comparison between groups and staining in the standard protocol of hematoxylin and eosin for stereological verification of muscle tissue. The observed results indicated time-dependent ergogenic improvements and slight reductions in the visceral fat of the supplemented animals, as well as variations of mononuclear phagocytes present in the tissue of the experimental groups, especially a low amount of these cells in the animals that trained and supplemented. It is concluded that creatine may have effects on visceral fat and the potential modulating effect of the innate immune system, in addition to time-dependent ergogenic effects.

Keywords: Creatine; Exercise; Fats; Muscle Strength; Performance-Enhancing Substances.

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INTRODUÇÃO

Physical exercise is recognized as a non-pharmacological intervention to help treat chronic cardiovascular and metabolic diseases. Nevertheless, many studies have observed positive effects of regular physical activity on neurodegenerative, psychological, osteometabolic and social conditions ^[1-6].

One of the organic systems that benefit most from regular physical exercise is muscle, and the skeletal striatum is of interest to this study. The musculoskeletal system is considered fundamental for many physiological processes, besides being essential to the locomotor system, since it is the active component of the human biological lever system ^[7]. Maintenance of homeostasis is also dependent on the adequate activity of the skeletal muscle system ^[8, 9], with evidence that injury or malfunction of this system can cause severe organ problems such as neurodegenerative ^[3] or osteometabolic diseases ^[2]. There is now a consensus that well-oriented and adjusted physical activity and exercise for each practitioner to enhance organic functions ^[1,2]. Improved muscle endurance and increased strength are variables that exemplify the positive effects of frequent physical exercise on the musculoskeletal system ^[10,11]. These factors, which, when well developed, contribute to the practitioner's health and mitigate the risk of developing or progressing chronic degenerative diseases ^[5,12].

Thus, the use of some dietary supplements may potentiate the gains of these variables, among the most studied by science and described in the literature is creatine ^[13]. A naturally occurring amine in eukaryotic cells, being endogenously synthesized from the amino acids arginine, methionine and glycine ^[14]. Its metabolism occurs through complementary reactions, both in the kidneys and pancreas, from the action of enzymes such as glycine amidinotransferase (GATM) and guanidinoacetate methyltransferase (GAMT), respectively ^[15].

The ergogenic effects of creatine use have already been documented amplification ^[13-17]. As an example of this, it was verified, through a systematic review and meta-analysis of the literature, the effects of creatine supplementation and changes in strength and performance in the upper limbs, in different ages, sports, physical conditions, and sex. The results obtained indicated that creatine use is better used when it is intended for a short duration and high-intensity exercises, due to the favor of the bioenergetic pathway of phosphocreatine, from the supplement administration, regardless of the age or gender of the practitioner ^[13, 18].

This is because creatine monohydrate, which is, which can be consumed exogenously and the most commercially available, increases the transport of phosphate from the interior of mitochondria to the sarcoplasmic environment, thus facilitating the synthesis of adenosine diphosphate in adenosine triphosphate ^[14].

¹⁷. Nevertheless, there are indications that exogenous creatine consumption aids in buffering intracellular hydrogen ions, which reduces hydrogenic potential, thereby increasing muscle endurance and alleviating post-training pain ^[13]. However, this last inference is still being studied and little is known for certain about it.

Another little known aspect of creatine use as a dietary supplement is its relationship with visceral (epididymal) fat. Some investigations have observed that reductions in this fat in obese animals are associated with improved insulin resistance ^[18,19]. However, investigations did not extend to the use of dietary supplements such as creatine, which is an important gap in the knowledge about adipose tissue, exercise, and supplementation.

Given this scenario, this study aims to analyze the effects of creatine supplementation on adipose, musculoskeletal tissue and physical performance variables in *Rattus norvegicus*.

MATERIAL AND METHOD

This study was approved by the Animal Research Ethics Committee of the Jundiaí School of Medicine under opinion number 490/12. It is worth noting that all procedures applied followed the protocols of organizations of animal good treatment and respect to experimental models, written by national and international regulatory and research ethics entities.

Twenty-four animals of the species

Rattus norvegicus var. *Albinus*, rodent phylum and mammalian class, kingdom *Mammalia*. These animals were organized into four groups, each with six animals. Group one was called control (G1), receiving exclusively food and water *ad libitum* 24 hours a day and 12/12 light / dark cycle. Group two received the same dietary treatment as the first group, however, three times a week (Mondays, Wednesdays, and Fridays), during the 25 weeks of the experimental protocol, received a supplementation of 0.3g of creatine monohydrate (brand Universal LTDA) per kilogram of body mass (via gavage), being called supplement group (G2). Group three, like the other groups, had the same diet and sleep pattern and lightness, but practiced on Monday, Wednesday and Friday, during the experimental period, a strength training protocol, with progressive load increase, based on the protocol of Horneberger and Farrar (2004), being called as training group (G3). Group four received treatment from group G2 in association with group G3, thus establishing a group that made use of supplement and training during the period of 25 training sessions, receiving the denomination of G4.

After the experimental period, the animals rested for 48 hours and were anesthetically induced with ethoxyethane, promoting central nervous system depressant action. Sequentially they were euthanized in CO₂ equipment, thus avoiding any suffering to the animal. Visceral fat was extracted and weighed immediately on an analytical balance

(Gehaka, model AG200), weighing results were recorded and computed using statistical analysis software (BioStat version 5.3).

The training loads of the groups that underwent the protocol were noted and subsequently computed using data analysis software (BioStat LE 6.9.1.0). The same procedure occurred with the climbs performed by the animals. Climbs considered adequate, that is, that started at the first step of the climbing platform (Honenberg and Farrar, 2004) and concluded at the last, were considered valid and noted. Climbs, where the animal failed between the first and last, were discarded and the successful climbing load was noted and then computed in the data analysis software. The stereological analysis followed the Harris and Eosin Hematoxylin staining protocol, as well as analysis of tissue components and their morphologies under 10, 40 and 100X objectives.

The statistical treatment employed was one way ANOVA with a t-student test for verification, assuming $p < 0.05$. All statistical treatment occurred in BioStat software (LE version 6.9.1.0).

RESULTS

Physical performance variables such as relative load and number of climbs, on average, were initially collected, since during the training program they were frequently updated according to the animal's success or failure during the exercise program. In this sense, graph one expresses the results verified in the relative strength variable, while graph two shows the average number of climbs performed by the animals in a comparison between the groups that performed the training protocol.

Graph 1: Relative burden of the groups that performed the training protocol. Groups III and IV had their loads noted and each was checked for animal body weight after this procedure the means were expressed in the graph. The * indication represents statistical difference by the t student test assuming $p < 0.05$. The vertical line shows the kilograms of the load and the horizontal the training days.

Graph two expresses the behavior of the climbs throughout the training program sessions. The comparison between

the groups is expressed on average, with the respective standard deviations assumed.

Graph 2: number of climbs of animals from groups III and IV of the experiment (average). Progression over 25 training sessions. Artificial mean obtained by BioStat software (LE version 6.9.1.0), from the descriptive analysis of the number of climbs of each animal belonging to the groups that performed the training protocol. The averages of each session were compared, with the symbol * indicating that group IV had a statistically different value compared to group III, while the symbol # indicates that group III had a statistically differentiated value compared to group IV. Comparative analysis by Anova one way, assuming $p < 0.05$.

Once the behavior of the loads and it is possible to comparatively quantify all these climbs over the training weeks has been verified, variables at the end of the training protocol.

Graph 3: Total burden of the groups that performed the training protocol. Relative load corrected for the bodyweight of the animals in this study. Statistical analysis ANOVA one way assuming $p < 0.05$ and with verification test for each group using Bonferroni, assuming $p < 0.03$. There was no statistical difference between the groups.

Graph 4: Total burden of the groups that performed the training protocol. Relative load corrected for the bodyweight of the animals in this study. Statistical analysis ANOVA one way assuming $p < 0.05$ and with verification test for each group using Bonferroni, assuming $p < 0.03$. There was no statistical difference between the groups.

The body weight of the animals was measured at the beginning of the study and the end of the experimental period, imme-

diately before euthanasia. Thus, graph five shows the comparison between body weight before and after the experimental protocol.

Graph 5: modification of bodyweight of animals throughout the experimental protocol. Comparison of the beginning and end of the experiment. Means obtained by the descriptive analysis performed in BioStat software (LE version 6.9.1.0). There was no statistical difference between the groups.

Graph six, in turn, expresses the results obtained from the weighting of visceral fat of animals from experimental groups and study controls. It is noteworthy that the weigh-

ing of this region occurred after euthanasia, which followed the recommendations of good treatment and ethics in animal research.

Graph 5: The average weight of the visceral fat of the experimental animals. Mean obtained by the descriptive analysis performed in the BioStat software (LE version 6.9.1.0). In the comparative analysis of the means, through Anova one way, assuming $p < 0.05$. The symbol * indicates the statistical difference between group II and all other groups in the experiment.

The results observed after the experimental period indicated that the creatine in association with the training presented strength increase of the animals that were in group four during the fourth to the fourteenth week of the protocol. However, the climbing results were not similar. Groups three and four often had similar climbing rates, and at the end of

the program the total sum of average climbs and total group load was close (graph 3 and 4).

Graph six illustrates the stereological observations obtained from light microscopy analysis. In this graph, it is possible to identify the relationship between the number of myocytes, fibroblasts and mononuclear phagocytes present in the tissue, according to the objective and the scale used.

Graph 6: Relationship between parenchyma and stroma (with different cell types present) of experimental groups in latissimus dorsi muscle. The averages obtained were expressed as percentages of the analyzed tissue. The statistical treatment used was Anova one way, assuming $p < 0.05$, with Bonferroni verification test, assuming $p < 0.03$. There were no statistical differences between the groups.

Stereological analysis was followed by image capture to observe the morphology of

cells present in muscle tissue. In this sense, figure one presents exemplifies this condition.

Figure 1: Stereological analysis of musculoskeletal tissue (latissimus dorsi muscle) of the experimental groups. Table A represents an animal from the control group. Table B represents the creatine group. Table C represents the training group and Table D represents the associated training and creatine group. Arrows indicate interphase nuclei of muscle tissue. Arrowheads have mononuclear phagocytes present in the stroma indicated by the letter S of the tissue. The letter F indicates the muscle fiber sarcoplasm and the letter E the endomysium that lines each fiber. The scale is standardized at 50.00µm per centimeter. The objective used was 10X.

DISCUSSION

The present study aimed to analyze the effects of creatine supplementation on adipose, musculoskeletal tissue and physical performance variables of *Rattus norvegicus*. In this sense, the literature shows that creatine supplementation is associated with an improvement in muscle strength production capacity, resulting in in-

creased muscle fiber volume, thus characterizing a hypertrophic condition^[18,19]. Creatine use for ergogenic improvement is widely documented, for example, in studies by Gualano et al, which found that creatine is best used when a person taking this supplement has a recovery time greater than 60 seconds. between resistance training series^[14]. Also, in another study, tissue morphometry was observed after creatine use in association with

training^[19]. Results indicated nuclear and sarcoplasmic morphometric differences for trained and supplemented animals when compared to only supplemented or only trained animals^[19].

Creatine interference in the cellular context occurs in the phosphocreatine bioenergetic pathway, muscle cell sarcoplasmic and hepatocyte cytoplasm^[1-4]. This bioenergetic pathway is closely associated with high intensity and short duration sports such as Olympic weightlifting and 100 meters of track and field^[20]. However, the phosphocreatine pathway is not restricted to sports and physical activity alone^[21]. People in the aging process are increasingly dependent on this bioenergetic pathway, given the need for strength production by the postural muscles and lower limbs for locomotion of this population^[22,23].

Thus, it is understood that checking the tissue effects of creatine, as well as other dietary supplements, are important aspects of nutrition and human health. In this sense, the present study sought to corroborate the existing tests in understanding the effects of creatine on muscle strength^[13,14,29]. However, as it was found, this intention was added to the search for identification of the effects on muscle resistance and visceral fat rate, factors that are still little studied^[13]. Graph one of the present study showed that the muscular strength of the animals had a variable and progressive behavior throughout the 25 training sessions, as can be observed from the second session in which there is already an increase in the relative load for both groups. performed the training protocol. Group IV, which was supplemented and trained, obtained statistically differentiated relative loads to group III, only trained, in the fourth, fifth and sixth sessions.

This increase in relative load is believed to be due to the use of creatine monohydrate in the animals of the four group^[14,17,23]. Creatine is known to be the major phosphate transporter in the cytoplasmic medium, facilitating the interaction of this element with adenosine diphosphate, thereby resynthesizing adenosine triphosphate^[21]. The result of this facilitated

resynthesis is a greater supply of the highly energetic molecule for cellular activities^[16]. Some studies, such as Fernandes et al, Galvan et al, and Gualano et al, have identified the potential effects of creatine association on strength exercise, which is much more expressive, in morphological, histological and molecular perspectives, than use. supplement only without concomitant training. In this sense, the results observed in this study corroborate the observations already available in the scientific literature^[17,19,23].

Still in an analysis of graph one, it is observed that the relative load results were more expressive for group four during the initial training sessions, while at the end of the sessions, around the 19th session, the results of the groups practically overlap. Kreider et al identified in their systematic review that creatine has better ergogenic effects in the initial weeks of use, and for unknown reasons, after a certain period of use, the loads of practitioners who use are equivalent to those who do not use. of the supplement^[13]. Some hypotheses arise from this condition observed in the literature and confirmed in this study. The most evident and accepted today is that intracellular creatine reserves reach a physiological limit, about 160mmols^[23], which leads to the excretion of surplus through the urinary tract^[15]. Another widely discussed hypothesis is the time-dependent relationship of creatine performance in ATP synthesis^[13]. It is already well accepted that the ideal recovery time is between 60 and 90 seconds of recovery between sets of strength training exercises for practitioners who use creatine monohydrate^[13,14]. Thus, prolonged use of the supplement and recovery time outside the optimal period does not result in substantial and statistically significant ergogenic improvements.

As for the number of climbs, which is similar to the number of repetitions for human conditions in a training program, the animals in groups three and four started the first two sessions with a very similar average, being five and six on average for the training and training and supplement groups, respectively. However, from the

third week on, group four had a statistically higher and significant average compared to group three. The session number nine presented an average of nine climbs for the training and supplement group and remained at five for the training only group. This result complements the observations of relative strength, presented in graph one and widely discussed in this session. Thus, it is clear that musculoskeletal resistance can be favored in a time-dependent manner with the use of creatine monohydrate, in addition to the development of strength^[13]. This finding, in association with those already available in the literature, suggests with confidence that creatine may be a supplement used in the treatment of muscular and degenerative diseases since its capacity to improve muscle strength and endurance seems to be effective.^[23,24] Riesberg et al^[25] found that creatine has properties that go beyond those mentioned above. The authors identified that creatine used in addition to the treatment of some degenerative diseases helped slow the progression of the pathology, a fact that is especially noticeable in neural and muscular diseases^[24].

The results observed in this study, regarding the behavior of mononuclear phagocytes, did not indicate statistically significant differences with creatine use or nuclear pleomorphism of these cells. However, they could have clinical relationships that are relevant to mention. According to graph six, in the percentage variable of mononuclear phagocytes, it can be verified that there was a slight increase of these cells in the only trained group, about 11% of the cells present in muscle tissue in this condition. It is noteworthy that the animals, regardless of the experimental group, were euthanized after 48 hours of the last training session and creatine consumption, thus avoiding any expression of molecules that could confuse the analysis of these cells, given the inflammatory potential of the training force possesses^[16]. However, when compared to the creatine groups, the phagocyte percentile was very similar to the control group and may indicate a potential

anti-inflammatory effect of creatine, which had been previously described in the literature^[13,25].

It is still sought to relate the attenuation effects of creatine hydrogen potential and its contributions to fight inflammation, since it is speculated that its administration would help to transport the hydrogen ions from plasma more easily, reducing the catalytic activity of mononuclear phagocytes and sensitivity to local pain^[13,25]. Therefore, with the information found in this study, the hypothesis that creatine has anti-inflammatory properties, although small, can be reinforced, since an increase of these cells was observed in animals that underwent the training protocol, but did not occur. The same is true for animals that trained and consumed the supplement, as shown in graph six.

The unpublished finding of the study, which contributes significantly to the scientific literature on this subject is the relationship between creatine use and visceral fat in animals. Graph four indicates that the average body mass increase behavior of the experimental groups was quite similar. However, graph five indicates that the group only supplemented with creatine, group two, obtained the smallest amount of visceral fat mass to all other animals of the experiment. This result is very promising in understanding the relationships between the use of this supplement for overweight, obese or bedridden people, since the total body mass of group two animals remained increasing, however, without increasing, when compared to all other groups in the experiment, the visceral fat of the animals^[28].

This relationship between creatine supplementation and its effects on adipose tissue had already been observed and it was found that the enzyme glycine amidinotransferase (GATM), besides being responsible for endogenous creatine synthesis in the cytoplasmic environment of renal cells, is also present in adipocytes and contributes to mitochondrial renewal of ATP, being a possible stimulator of mitochondrial thermogenesis, which increases caloric consumption^[30]. In this sense, creatine supplementation

could increase this ATP renewal, since oxygen consumption per kilogram of body mass is influenced by animals that supplemented creatine [30]. In this study, in addition to literature data, it was found that the reduction of visceral fat was more evident in group II, only supplemented with creatine. The fact that is noteworthy is that group four, which made use of the supplement in association with training, did not obtain any minor statistical difference to the control group in the variable visceral fat. The question about this association and a possible loss of creatine thermogenic capacity is solved by the observation of higher dietary intake of animals from group IV compared to group II since the practice of strength training increases ghrelin stimulation and control of leptin [31]. In this sense, it would be likely that animals that consumed more food, due to hunger caused by strength training, increased visceral fat in a controlled manner. Moreover, it is noted that the association of both conditions did not alter the behavior of phagocytic cells in muscle tissue, according to the stereological analysis and graph six, being, in this respect, a beneficial association, since it maintains the Visceral fat statistically comparable to the control group increases muscle strength and endurance and contributes to positive maintenance of resident phagocytic cells of muscle tissue.

It can be seen from the results obtained in this study and the literature currently available and consolidated on the research theme, that creatine has better availability of energy synthesis from the second to the sixth training session, and also observed an improved muscle endurance from the second to the ninth session. Furthermore, it is found that creatine does not induce visceral fat mass increase and is possibly able to attenuate this variable since it can increase muscle volume without affecting adipose tissue [23, 30, 31]. Moreover, the association between supplementation and training is the best way to use this substance to promote ergogenic effects and potential post-training tissue repairs, since the number of mononuclear phago-

cytes remains similar to the control group [14].

CONCLUSION

Creatine had ergogenic effects associated with strength training in animals submitted to both conditions. Visceral fat in animals only supplemented with creatine was lower than visceral fat in the other groups, even maintaining a similar body mass. Creatine not only improves muscle strength during the first few training sessions but also increases average climbing. These factors progressively stabilize according to the number of sessions held. Creatine has anti-inflammatory effects capable of assisting in tissue repair after the training session, however, for this variable further studies are needed.

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