EFFECT OF CREATINE AND SILDENAFIL CITRATE ON THE PHYSICAL PERFORMANCE OF MICE

EFEITO DA CREATINA E DO CITRATO DE SILDENAFILA SOBRE O DESEMPENHO FÍSICO DE CAMUNDONGOS

EFECTO DE LA CREATINA Y DEL CITRATO DE SILDENAFIL SOBRE EL DESEMPEÑO FÍSICO DE RATONES

ORIGINAL ARTICLE
ARTIGO ORIGINAL
ARTÍCULO ORIGINAL

Wendel Simões Fernandes[1]
(Biochemical Pharmacist)
Rodolfo de Paula Vieira[1]
(Physical Education Professional)
Ricardo César Alves Ferreira[1]
(Physical Education Professional)
Sérgio César Ferreira[1]
(Physical Education Professional)
Viviane Gadret Bório Conceição[2]
(Pharmacist)
Flávia Villaça Morais[3]
(Biomedical Scientist)
Aline Neves Araújo[2]
(Biologist)
Priscila Ebram de Miranda[2]
(Biochemical Pharmacist)
Patrícia Destefano[1]
(Physical Education Professional)
Wellington Ribeiro[4]
(Pharmacist)

1. Universidade Federal de São Paulo, Graduate Program in Human Movement and Rehabilitation Sciences, Santos, SP, Brazil.
2. Universidade Paulista, Instituto de Ciências da Saúde, São José dos Campos, SP, Brazil.
3. Universidade do Vale do Paraíba, Instituto de Pesquisa e Desenvolvimento, Cellular and Molecular Fungal Biology Laboratory, São José dos Campos, SP, Brazil.
4. Universidade do Vale do Paraíba, Instituto de Pesquisa e Desenvolvimento, São José dos Campos, SP, Brazil.

Correspondence:
Wendel Simões Fernandes. Rua Talim, 330, São José dos Campos, SP, Brasil. 12231-280.
wen_sfernandes@hotmail.com

ABSTRACT

Introduction: The use of substances to enhance sports performance among professional and amateur athletes is increasing. Such substances may either be included in the group of dietary supplements or fall into pharmacological classes. Every substance used for this purpose is called an ergogenic agent. The number of ergogenic options available increases every day, favoring overuse and use without proper guidance. Among the dietary supplements, we highlight the use of creatine, a substance widespread in sports. Among the pharmacological groups, many drugs are used. Recently the use of sildenafil citrate by professional athletes from various predominantly aerobic sports modalities was reported in the media. Objective: To compare and demonstrate the responses caused by physical training associated with the use of creatine and sildenafil citrate in mice. Methods: A swim training protocol was applied and then an electrophysiograph was used in order to obtain parameters related to contraction intensity, the area under the curve and the percentage drop. Results: The responses obtained demonstrated the ergogenic action of creatine because it altered the parameters used for measurement. The use of sildenafil citrate did not yield satisfactory results to frame the drug as an ergogenic agent. Conclusion: Creatine has an ergogenic effect, reducing the percentage drop after 10 seconds, while sildenafil demonstrated no ergogenic potential and, interestingly, resulted in weaker responses when compared to the exercise groups. ***Evidence level II; Comparative prospective study.***

Keywords: Creatine; Muscle fatigue; Sildenafil citrate.

RESUMO

Introdução: O uso de substâncias com o objetivo de aumentar o rendimento esportivo entre atletas profissionais e amadores é crescente. Tais substâncias podem fazer parte do grupo de suplementos alimentares ou integrar classes farmacológicas. Toda substância empregada para esse fim é denominada de agente ergogênico. O número de opções entre os agentes ergogênicos aumenta a cada dia, favorecendo assim o uso em demasia e sem a devida orientação. Entre os suplementos alimentares, salientamos a utilização de creatina, substância muito difundida no meio esportivo. Já entre os grupos farmacológicos, muitas substâncias são utilizadas. Recentemente, foi divulgado entre os meios de comunicação o uso de citrato de sildenafila por atletas profissionais de várias modalidades esportivas, predominantemente as aeróbicas. Objetivos: Comparar e demonstrar as repostas ocasionadas pelo treinamento físico, associadas ao uso de creatina e citrato de sildenafila em camundongos. Métodos: Aplicou-se um protocolo de treinamento de natação e, a seguir, empregou-se um eletrofisiógrafo com objetivo de obter parâmetros referentes à intensidade de contração, à área sob a curva e à queda percentual. Resultados: As respostas obtidas demonstram ação ergogênica da creatina, visto que alteraram os parâmetros empregados para a mensuração. Já a utilização de citrato de sildenafila não apresentou resultados satisfatórios para enquadrar o fármaco como agente ergogênico. Conclusão: A creatina apresenta efeito ergogênico porque reduz a queda percentual após 10 segundos, já a sildenafila não apresentou potencial ergogênico e, curiosamente, demonstrou respostas inferiores quando comparado aos grupos de exercício. ***Nível de evidência II; Estudo prospectivo comparativo.***

Descritores: *Creatina; Fadiga muscular; Citrato de sildenafila.*

RESUMEN

Introducción: El uso de sustancias con el objetivo de aumentar el rendimiento deportivo entre atletas profesionales y amateurs es creciente. Tales sustancias pueden formar parte del grupo de suplementos alimentarios o integrar clases farmacológicas. Toda sustancia empleada para ese fin es denominada agente ergogénico. El número de opciones entre los agentes ergogénicos aumenta cada día, favoreciendo así su uso excesivo y sin la debida orientación. Entre los suplementos alimentarios, se destaca el uso de creatina, sustancia muy difundida en el medio deportivo. Ya entre los grupos farmacológicos, muchas sustancias son usadas. Recientemente, fue divulgado entre los medios de comunicación el uso de citrato de sildenafil por atletas profesionales, de varias modalidades deportivas, predominantemente las aeróbicas. Objetivos: Comparar y demostrar las respuestas ocasionadas por el entrenamiento físico,

asociadas al uso de creatina y citrato de sildenafil en ratones. Métodos: Se aplicó un protocolo de entrenamiento de natación y, a continuación, se usó un electrofisiógrafo con el objetivo de obtener parámetros referentes a la intensidad de contracción, al área bajo la curva y a la caída porcentual. Resultados: Las respuestas obtenidas demuestran acción ergogénica de la creatina, visto que alteraron los parámetros empleados para la medición. Ya el uso de citrato de sildenafil no presentó resultados satisfactorios para encuadrar al fármaco como agente ergogénico. Conclusión: La creatina presenta efecto ergogénico porque reduce la caída porcentual después de 10 segundos, mientras que el sildenafil no presentó potencial ergogénico y, curiosamente, demostró respuestas inferiores cuando comparado a los grupos de ejercicio. ***Nivel de evidencia II; Estudio prospectivo comparativo.***

Descriptores: *Creatina; Fatiga muscular; Citrato de sildenafil.*

DOI: http://dx.doi.org/10.1590/1517-869220212702153173 Article received on 08/12/2015 accepted on 11/04/2020

INTRODUCTION

The use of substances to enhance physical performance and body aesthetics is growing. Such substances, called ergogenic, attract the attention of professional and amateur athletes with the aim of improving physical performance in competitions or simply for the aesthetic benefits.[1] Among the substances used by athletes, we can mention a few pharmacological classes, such as vasodilators, bronchodilators, steroid hormones and an extensive group of dietary supplements.[2] It is noteworthy that other ergogenic resources can be used, in addition to nutritional and pharmacological ones.

The number of ergogenic substance options grows daily and access to these resources ends up becoming relatively easy, both for athletes and their trainers.[3,4] Based on the growing number of compounds being used, we can observe the use of substances already known for this purpose, as is the case with creatine, but we can also find substances with other purposes being used as ergogenic ingredients, as is the case with sildenafil citrate, a medication used to treat erectile dysfunction.

The experimental protocol used consists of the isolation of the sciatic nerve and the tibial muscle of mice, and their subsequent connection to the electrophysiograph with controlled electrical stimulation. In animal models, the anterior tibial muscle is of significant interest for studies due to its relatively large size, for its innervation by the sciatic nerve, and for its mixed composition of muscle fiber types. Because of these characteristics, it has been widely used as a study tool.[5]

The decision to use an ergogenic agent without proper guidance is based on the supposed benefits that consumers themselves imagine achieving, ignoring the possible harm that this practice can cause.[6] In this way, the justification for this study is the elucidation of the ergogenic effects of both creatine, which is widely used in sports circles, and sildenafil, the use of which as an ergogenic substance, predominantly in aerobic modalities, was recently reported in the media.

Thus, the main objective of the study is to observe the changes caused by the use of creatine and sildenafil associated with an aerobic training protocol in mice.

METHODS

All the experiments were approved by the Ethics Committee on the Use of Animals in Research of the Universidade do Vale do Paraíba under protocol A04/CEUA/2014. The entire study was conducted at the Physiology and Pharmacodynamics Laboratory of the Research and Development Institute at the Universidade do Vale do Paraíba.

This is an experimental study involving male Swiss mice, submitted to swimming training with increased load. After the training protocol, the animals underwent a surgical procedure to isolate the sciatic nerve and the right tibial tendon, making it possible to connect the animal to the electrophysiograph. Using induced electrical stimulation, parameters related to contraction intensity, the area under the curve and the percentage drop after 10 seconds were obtained.

Animals

Twenty-four male Swiss mice from the Anilab Campinas vivarium, weighing between 25 and 30 grams were used. All animals went through a 2-week adaptation period at the Universidade do Vale do Paraíba vivarium before the start of the experiments. The animals were kept in cages lined with wood shavings and a maximum occupancy of 5 animals, with no restrictions on food and water, and in 12-hour light and dark cycles.

Experimental Groups

The animals were divided into four groups, containing six animals per group, as follows:

- Group (sedentary): sedentary animals, no supplements, food and water maintained.
- Group (swimming): animals submitted to swim training protocol, no supplements, food and water maintained.
- Group (swimming and sildenafil): animals submitted to swim training protocol, receiving the drug sildenafil citrate (10 mg/kg) during the entire training protocol, food and water maintained.
- Group (swimming and creatine): animals submitted to swim training protocol, receiving the drug creatine (1 g/kg) during the entire training protocol, food and water maintained.

Administration of Sildenafil Citrate

The sildenafil citrate was administered by intraperitoneal injection daily during the entire experiment, 30 minutes before conducting the training protocol, based on the peak of action of the substance.[7] The drug was obtained in crude raw material form (PharmaNostra, lot 0500520115c, São José dos Campos, SP). For administration, the drug was solubilized in saline to produce a solution. The dose used was 10 mg/kg.[6]

Administration of Creatine

The creatine was administered orally, using a gavage needle, daily 30 minutes before the protocol was conducted. The supplement was obtained in its commercial monohydrate form (Universal Nutrition, USA, lot 186167b). The administration scheme was divided into two stages; the first week of the experiment consisted of a loading phase, in which the dose was 5 g/kg of body mass of the animal and the second stage was called the maintenance phase, with a dose of 1 g/kg of animal body mass.[8] The creatine was solubilized in water to facilitate administration to the animal.

Training Protocol

The training protocol used consisted of 30-minute swimming sessions, 5 times a week for 6 weeks. The training was conducted in containers with approximate dimensions of 100 cm x 70 cm x 50 cm filled with previously heated and temperature-monitored water (30-34 °C). The animals went through a 2-week adaptation period to minimize effects related to the stress that exercise could cause them. After the adaptation period, all the animals were submitted to the protocol, with a

weight cell at the base of the tail corresponding to 70% of the maximum load supported by each animal. The weight was determined using the maximum load test. The time spent in the water was carefully monitored by chronometer and no animal exercised beyond that stipulated in the protocol. After the sessions, all animals were towel-dried manually and returned to their respective containment boxes.

Maximum Load Test

The maximum load test was performed individually, with load cells corresponding to 1%, 2%, 3% etc. of the weight of the animal, submitting it to 3 minutes of swimming and 1 minute of recovery before increasing the load, until its exhaustion, thus determining the maximum load supported. Exhaustion of the animal was determined by its inability to remain at the surface.[8] The maximum load test was essential to determine the swim training load, which was 70% of the maximum load supported, the value used in training.[9]

Anesthesia Protocol

The animals were anesthetized using Xylazine 2% and Ketamine 10% via intraperitoneal injection. During anesthetic induction, the animals were monitored and kept warm with the constant presence of light. Immediately after the loss of reflexes was observed they underwent the surgical procedure.

Surgical Procedure

The surgical procedure aimed to isolate the sciatic nerve and the tibial tendon, connecting them respectively to electrodes and to the isometric transducer connected to the physiograph. With the anesthetized animal positioned on the operating table, a longitudinal skin incision was made in the anterior surface of the tibial region, under which the tibial tendon is located. The tendon was isolated and connected to the isometric transducer (UGO BASILE). The sciatic nerve was isolated through an incision in the muscle and connected to the dipole electrode (UGO BASILE). The tibial muscle was submitted to a constant tension of 1g and it was stimulated indirectly by a 4V, 0.2 Hz pulse 2 milliseconds in duration for contraction. For 3 minutes the contraction was maintained in a serial manner, after which a 10-second tetanic contraction was induced. This process was repeated two more times. To induce the tetanic contraction the frequency was increased to 50 Hz. At the end of the procedure, we had obtained 3 tetanic contractions for each animal. All contractions were recorded by the UGO BASILE GEMINI 7070 physiograph. In animal models, the anterior tibial muscle is of significant interest for research due to its relatively large size, its innervation by the sciatic nerve and its mixed composition of muscle fiber types, and because of these characteristics it has been widely used as a study tool.[5] The intensity of the contraction force was measured using a standard equivalent to 1.0 gram. The percentage drop was measured by determining the maximum peak of contraction and the peak existing after 10 seconds of tetanic contraction. The area under the curve (AUC) was determined by calculating the area of the trapezoid according to the following reasoning: It is known, by the reasoning of differential and integral calculus, that if f(x) is a continuous function in the interval [a,b], then this function has a primitive in it, i.e., there exists an f(x) such that f'(x)=f(x). To resolve the problem of calculating the approximate area under a certain curve we have the trapezoidal rule. The concept of the trapezoidal rule is to approximate function f(x) with a first-degree polynomial (straight). We can see, in this approximation, that the integral of the function f(x) can be approximated by the area of the trapezoid.[10]

Euthanasia

The animals were euthanized through intracardiac injection of the chemical agent potassium chloride (KCl) 20% in solution. The euthanasia procedure was performed with the animals still anesthetized.

Statistical Analyses

One-way ANOVA was used, followed by the Tukey-Kramer multiple comparison test. A p-value less than 0.05 was considered a statistically significant difference.

RESULTS

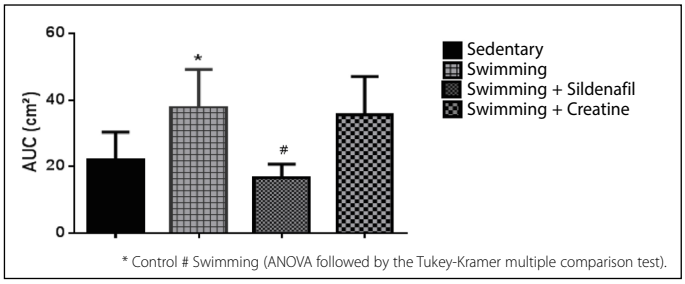

Figure 1. Area under the curve. The data are presented as the mean ± standard deviation. n= 6, p<0.05 when compared with the response obtained for the groups

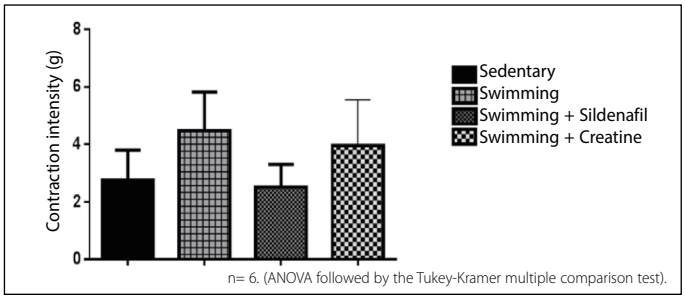

Figure 2. Contraction Intensity. The data are presented as the mean ± standard deviation.

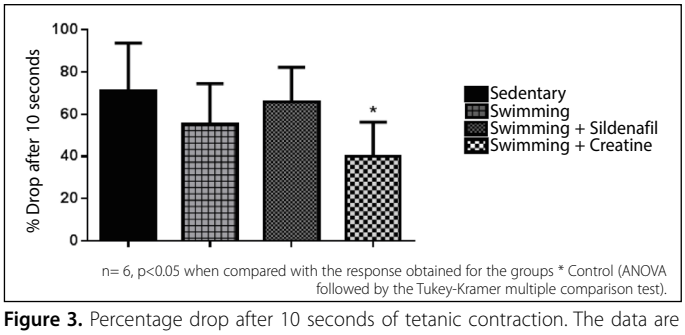

Figure 3. Percentage drop after 10 seconds of tetanic contraction. The data are presented as the mean ± standard deviation.

DISCUSSION

The effects caused by physical exercise applied through continuous training protocols are described in the literature. Structural and metabolic changes in the musculoskeletal tissue may result in responses of increased performance, causing a possible increase in contraction strength, contraction amplitude and resistance to fatigue.[11,12] The question to be discussed is the response generated through the association of physical exercise with possible ergogenic resources, in the cases of creatine and sildenafil citrate, which are the targets of this research. The results found demonstrate the ergogenic effect of creatine, but refute the possible ergogenic effect attributed to sildenafil.

Analyzing the responses obtained for the area under the curve (Figure 1), we see a significant difference between the control and the swimming groups, strengthening the reasoning that changes were caused by the physical training. These changes appear to be related to neural factors and to muscle hypertrophy that occurs when the exercise is performed with increased weight.[13]

The exercise protocol carried out in this study uses the overload principle, based, therefore, on inducing a disturbance in the homeostasis

of the animal to which the organism responds in a positive way, a process known as positive physiological adaptation.[14] When the recovery time between the exercise session is respected, positive adaptation occurs through the metabolic and morphological remodeling of the myofibrils mediated by gene activation, which results in changes in the muscle mass.[15,16]

There is a high area under the curve that we must consider, even though it was not statistically significant when we analyzed the swimming associated with the use of creatine group, that can be interpreted as a response to the physical training performed by these animals. Therefore, the high result of this parameter should not be attributed to the use of creatine, but rather to the training protocol performed. The same logic is not used to justify the results of the swimming associated with sildenafil group, since the animals went through the same training protocol and presented much lower responses compared to the other exercised groups.

The parameter determined by contraction intensity (Figure 2) did not present statistically significant differences, but one can see a trend towards better results in the swimming and swimming associated with the use of creatine groups. We attribute these results to the positive adaptations caused by physical exercise, in contrast to the conclusions found in a study that attributes creatine with the potential to improve muscle strength performance, though in programs focused on muscle strength.[17]

The swimming group associated with the use of sildenafil had results very similar to those of the sedentary group. However, these results corroborate the results presented in a study that reported that sildenafil significantly decreased running capacity in a test conducted with rats, in which the decrease intensified as the dose of the drug was increased.[7]

The parameter involving the percentage drop after 10 seconds of tetanic contraction (Figure 3) is directly linked to resistance and muscular fatigue, and the group with the lowest drop showed increased resistance to fatigue, in this study the swimming associated with the use of creatine group. This increase can be attributed to the creatine phosphate (CrP) replacement mechanism in the muscle cells caused by the ingestion of creatine. When physical exercise is performed for a certain amount of time, ATP levels are reduced and physiologically recomposed through the CrP molecules, but if the physical stimulus is maintained, the stock of CrP is reduced.[18,19,20]

According to studies conducted involving the ingestion of creatine in two phases, one of loading and the other of maintenance, a 20% increase in muscle creatine levels was found.[21,22] This fact could favor maintaining the CrP levels and improve the fatigue threshold.

Another factor attributed to creatine that may justify the improvement in the fatigue threshold is the buffering effect caused by the CrP molecule. In the conversion of ADP into ATP through CrP hydrogen ions are consumed, thus increasing the local pH and mitigating the negative effects that acidosis can have on muscle performance.[23,24]

Again, the swimming associated with the use of sildenafil group did not produce results that can qualify the substance as an ergogenic agent, corroborating a study conducted with athletes in which it was demonstrated that at normal altitudes sildenafil is not able to improve sports performance, but at high altitudes the drug tends to improve performance because in these situations a hypoxic condition occurs and, therefore, the vasodilation induced by using the drug is advantageous for better physical results.[25,26,16]

In this way, the results demonstrate the ergogenic action of creatine, acting to increase the fatigue threshold. Sildenafil did not improve sports performance, yielding results very similar to those of the sedentary group.

CONCLUSION

The results presented demonstrate that creatine can be interpreted as an ergogenic agent, since it interferes with sports performance and is effective in reducing the percentage drop after 10 seconds, thus favoring an increase in the muscle fatigue threshold. Although many studies have demonstrated the role of creatine in increasing strength, the results obtained in the present study do not support this change. The data involving the swimming associated with sildenafil group demonstrate that using the drug does not induce improvement in sports performance.

All authors declare no potential conflict of interest related to this article

AUTHORS' CONTRIBUTIONS: Each author made significant individual contributions to this manuscript. WSF: conception, study design, training and surgical procedures, data collection, interpretation of data, writing and review of the paper; RPV: interpretation of the data, writing and review of the paper; RCAF: conception, study design, training and surgical procedures, data collection, interpretation of data; SCF: interpretation of the data, writing and review of the paper; VGBC: interpretation of the data, writing and review of the paper; FVM: interpretation of the data, writing and review of the paper; ANA: interpretation of the data, writing and review of the paper; PEM: interpretation of the data, writing and review of the paper; PD: interpretation of the data, writing and review of the paper; WR: conception, study design, training and surgical procedures, data collection, interpretation of data, writing and review of the paper and guidance for the entire experimental protocol.

REFERENCES

1. Fontana KE, Valdes H, Baldissera V. Glutamina como suplemento ergogênico. Rev Bras Ciênc Mov. 2003;11(3):91-6.
2. Souza R, Ceni GC. Uso de suplementos alimentares e autopercepção corporal de praticantes de musculação em academias de Palmeiras das Missões – RS. Rev Bras Nutr Esport. 2014;8(43):20-9.
3. Molinero O, Marquez S. Use of nutritional supplements in sports: risks, Knowledge and behavioural- related factors. Nutr Hosp. 2009;24(2):128-34.
4. Ribas Filho D, Silva FBB, Botura RI. Avaliação do uso de ergogênicos por praticantes de academia no Noroeste e Nordeste no estado de São Paulo. Intern J Nutrology. 2014;7(2):05-10.
5. Shin RH, Vathana T, Giessler GA, Friedrich PF, Bishop AT, Shin AY. Isometric tetanic force measurement method of the tibialis anterior in the rat. Microsurgery. 2008; 28(6):452-7.
6. Santos MAA, Santos RP. Uso de suplementos alimentares como forma de melhorar a performance nos programas de atividade física em academias de ginástica. Rev Pau Educ Fís. 2002;16(2):174-85.
7. Rinaldi B, Donniacuo N, Sodano L, Gritti G, Signoriello S, Parreta E, et al. Effects of sildenafil on the gastrocnemius and cardiac muscles of rats in model of prolonged moderate exercise training. PlosOne. 2013;8(7):1-13.
8. Souza RA, Santos RM, Osório RAL, Cogo JC, Priante Júnior ACG, Martins RABL, et al. Influência da suplementação aguda e crônica de creatina sobre as concentrações sanguíneas de glicose e lactato de ratos wistar. Rev Bras Med Esporte. 2006;12(6):361-5.
9. Osorio RAL, Silveira VLF, Maldjian S, Morales Júnior A, Christofani JF, Russo AK, et al. Swimming of pregnant rats at different water temperatures. Comp Biochem Physiol A MolIntegr Physiol. 2003;135(4):605-11.
10. Ruggiero MAG, Lopes VL. Cálculo Numérico Aspectos Teóricos E Computacionais. São Paulo: Mackron Books; 1998.
11. Kraemer WJ, Ratmess MA. Hormonal responses and adaptations to resistance exercise and training. Sports Med. 2005;35(23):339-6.
12. Baltgalvis KA, Call JA, Cochrane GD, Laker RC, Yan Z, Lowe DA. Exercise training improves plantar flexor muscle function in mdx mice. Med Sci Sports Exerc. 2012;44(9):1671-9.
13. Dias RMR, Cyrino ES, Salvador EP, Nakamura FY, Pina FLC, Oliveira AR. Impacto de oito semanas de treinamento com pesos sobre a força muscular de homens e mulheres. Rev Bras Med Esporte. 2005;11(4):224-9.
14. Toigo M, Boutellier U. New fundamental resistance exercise determinants of molecular and cellular muscle adaptations. Eur J Appl Physiol. 2006;97(15):643-63.
15. Smith LL. Tissue trauma: the underlying cause of overtraining syndrome? J Strength Cond Res. 2004;18(6):185-93.
16. Walters J. Muscle hypertrophy and pseudohypertrophy. Pract Neurol. 2017;17(5):369-79.
17. 17. Corrêa DA, Lopes CR. Efeitos da suplementação de creatina no treinamento de força. Rev Bras Nutr Esportiva. 2014;8(45):180-6.
18. Wang CC, Fang CC, Lee YH, Yang MT, Chan KH. Effects of 4-Week Creatine Supplementation Combined with Complex Training on Muscle Damage and Sport Performance. Nutrients. 2018;10(11):1640.
19. Hummer E, Suprak DN, Buddhadev HH, Brilla L, San Juan JG. Creatine electrolyte supplement improves anaerobic power and strength: a randomized double-blind control study. J Int Soc Sports Nutr. 2019;16(1):24.
20. Sampaio CRSF, Aidar FJ, Ferreira ARP, Santos JLD, Marçal AC, Matos DG, et al. Can Creatine Supplementation Interfere with Muscle Strength and Fatigue in Brazilian National Level Paralympic Powerlifting? Nutrients. 2020;12(9):2492.
21. Stares A, Bains M. The Additive Effects of Creatine Supplementation and Exercise Training in an Aging Population: A Systematic Review of Randomized Controlled Trials. J Geriatr Phys Ther. 2020;43(2):99-112.
22. Volek JS, Kraemer WJ, Bush JA, Boetes M, Incledon T, Clark KL, et al. Creatine supplementation enhances muscular performance during high-intensity resistance exercise. J Am Diet Assoc. 1997;97(7):765-70.
23. Lambert CP, Flynn MG. Fatigue during high intensity intermittent exercise. Sports Med. 2002;32(8):511-22.
24. Gomes RV, Aoki MS. Suplementação de creatina anula o efeito adverso do exercício de endurance sobre o subsequente desempenho de força. Rev Bras Med Esporte. 2005;11(2):131-4.
25. Hsu AR, Barnhout KE, Grundmann NK, Lin JH, MacCallun SW, Frienlander AL. Sildenafil improves cardiac output and exercise performance during acute hypoxia, but not normoxia. J Appl Physiol. 2006;100(6):2031- 40.
26. Carter EA, Sheel AW, Milsom WK, Koehle MS. Sildenafil does not improve performance in 16.1 km cycle exercise time-trial in acute hypoxia. PLoS One. 2019;14(1):e0210841.