SHORT-TERM HIIT DOES NOT PROMOTE OXIDATIVE STRESS OR MUSCLE DAMAGE

HIIT DE CURTO PRAZO NÃO PROMOVE ESTRESSE OXIDATIVO OU DANOS MUSCULARES

HIIT DE CORTO PLAZO NO PROMUEVE ESTRÉS OXIDATIVO O DAÑOS MUSCULARES

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ABSTRACT

Introduction: High intensity interval training (HIIT) is a method that is widely used today. Objective: The present study aimed to evaluate the effects of HIIT on markers of oxidative stress and muscle damage in rats. Methods: The sample consisted of 60-day-old Wistar rats, divided into two groups: a control group (n=8) and an HIIT group (n=8). The training consisted of fourteen 20-second swimming sessions (loaded with weights equivalent to 14% of their body weight) with 10-second intervals between each session, performed for 12 consecutive days. Results: HIIT induced a reduction (–17.75%) in thiobarbituric acid reactive substances (an oxidative stress marker) in hepatic tissue (p=0.0482). There was also a reduction (–31.80%) in the HIIT group in the level of superoxide dismutase enzyme activity in the liver (p=0.0375). However, there were no differences between the groups in catalase, glutathione peroxidase, glutathione reductase, the total content of SH sulfhydryls, hydroperoxides, or carbonylated proteins in the hepatic tissue. No significant differences were found in any of these markers in the gastrocnemius muscle. The muscle damage markers creatinine kinase and lactate dehydrogenase were also similar between the groups in the gastrocnemius. Conclusion: The conclusion was that that short-term HIIT does not cause oxidative stress or muscle damage. ***Level of evidence I; High-quality randomized clinical trial with or without statistically significant difference, but with narrow confidence intervals.***

Keywords: High-Intensity Interval Training; Oxidative stress; Liver; Muscle.

RESUMO

Introdução: O treinamento intervalado de alta intensidade (HIIT) é um método muito utilizado atualmente. Objetivo: O presente estudo objetivou avaliar os efeitos do HIIT em curto prazo sobre marcadores de estresse oxidativo e dano muscular em ratos. Métodos: A amostra consistiu em ratos Wistar com 60 dias de idade, divididos em dois grupos: grupo controle (n = 8) e grupo HIIT (n = 8). O treinamento consistiu em quatorze sessões de natação de 20 segundos (com cargas equivalentes a 14% do peso corporal) com intervalos de 10 segundos entre cada sessão, realizadas por 12 dias consecutivos. Resultados: O HIIT induziu uma redução (-17,75%) das substâncias reativas ao ácido tiobarbitúrico (um marcador de estresse oxidativo) no tecido hepático (p = 0,0482). Houve também redução (-31,80%) no grupo HIIT no nível de enzima superóxido dismutase no fígado (p = 0,0375). No entanto, não houve diferenças entre os grupos com relação a catalase, glutationa peroxidase, glutationa redutase, teor total de sulfidrilas SH, hidroperóxidos ou proteínas carboniladas no tecido hepático. Nenhuma diferença significativa foi encontrada em qualquer um desses mascadores no músculo gastrocnêmio. Os marcadores de lesão muscular, creatinina quinase e lactato desidrogenase, também foram semelhantes entre os grupos no gastrocnêmio. Conclusão: Foi possível concluir que o HIIT de curta duração não causa estresse oxidativo ou dano muscular. ***Nível de evidência I; Estudo clínico randomizado de alta qualidade com ou sem diferença estatisticamente significante, mas com intervalos de informação estreitos.***

Descritores: *Treinamento Intervalado de Alta Intensidade; Estresse oxidativo; Fígado; Músculos.*

RESUMEN

Introducción: El entrenamiento en intervalos de alta intensidad (HIIT) es un método muy utilizado actualmente. Objetivo: El presente estudio tuvo como objetivo evaluar los efectos del HIIT en corto plazo sobre marcadores de estrés oxidativo y daño muscular en ratones. Métodos: La muestra consistió en ratones Wistar con 60 días de edad, divididos en dos grupos: grupo control (n = 8) y grupo HIIT (n = 8). El entrenamiento consistió en catorce sesiones de natación de 20 segundos (con cargas equivalentes a 14% del peso corporal) con intervalos de 10 segundos entre cada sesión, realizadas durante 12 días consecutivos. Resultados: El HIIT indujo una reducción (-17,75%) de las sustancias reactivas al ácido tiobarbitúrico (un marcador de estrés oxidativo) en el tejido hepático (p = 0,0482). También hubo reducción (~31,80%) en el grupo HIIT en el nivel de enzima superóxido dismutasa en el hígado (p=0,0375). Sin embargo, no hubo diferencias entre los grupos con relación a catalasa, glutatión peroxidasa, glutatión reductasa, tenor total de sulfhidrilos SH, hidroperóxidos o proteínas carboniladas en el tejido hepático. No fue encontrada ninguna diferencia significativa en ninguno de esos marcadores en el músculo gastrocnemio. Los marcadores de lesión muscular, creatinina quinasa y lactato deshidrogenasa también fueron similares entre los grupos en el gastrocnemio. Conclusión: Fue posible concluir que el HIIT de corta duración no causa estrés oxidativo o daño muscular. ***Nivel de evidencia I; Estudio clínico aleatorizado de alta calidad con o sin diferencia estadísticamente significativa, pero con intervalos de información estrechos.***

Descriptores*: Entrenamiento de Intervalos de Alta Intensidad; Estrés oxidativo; Hígado; Músculos.*

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INTRODUCTION

High intensity interval training (HIIT) is characterized by periods of very vigorous exercise interspersed with periods of absolute rest or low intensity intervals of active recovery.[1,2] During exercise there is an increase in the flow of oxygen to the skeletal muscles, which in turn favors the formation of reactive oxygen species (ROS).[3]

Oxidative stress (OE) is caused by an imbalance between oxidation/reduction reactions. The imbalance is caused by increased production of ROS that exceeds the tissue's antioxidant capacity. The excess of ROS favors oxidative reactions and, consequently, cell damage.[4,5] These events, along with post-exercise inflammatory responses, may result in muscle damage.[5] The degree of muscle damage and inflammation are proportional to the intensity of the exercise.[6]

In the protocol used by Terada et al.,[7] the animals were subjected to overloads equivalent to 14% of body weight and, according to the authors in this overload is already sufficient to superimpose an intensity of 80% of the animals'VO2max. Previous studies[8-10] identified that the use of this load is already considered to be of high intensity for rats. In addition, Ramos-Filho et al.[11] and Pimenta et al.[12] used the same protocol as Terada et al.[7] In this sense, the control of the training load is fundamental for the achievement of specific objectives, among them, the improvement of performance, that is, in the imbalance between volume, intensity and density in the training session, an increase in the concentration of species is visible. reactive oxygen (ROS), which can lead to oxidative stress.

One of the HIIT models is the low volume, which refers to relatively brief exercise training sessions covering less than or equal to 10 minutes of intense exercise in a training session with less than or equal to 30 minutes, including warm-up, recovery periods between intervals and cooling in the way that if compared with traditional public health guidelines, total weekly exercise and training time is reduced.[1] Previous studies[1,2] relatively small and short term suggest that HIIT can be as effective as traditional continuous training of moderate intensity to induce physiological remodeling, which in turn can be associated with improved health markers, despite a reduction in the duration of the exercise session. Although HIIT has become a popular training modality for athletes, as well as for the general population, there is still little information on oxidative stress related to short-term training. Therefore, little is known about the effects of HIIT on OS markers, and on the magnitude of some oxidative and muscle damage markers.

Some authors[6,13-15] who evaluated the effects of HIIT on markers of oxidative stress and muscle damage have presented, for the most part, evidence of the existence of intercalated recovery periods between sessions, but there is still a scientific gap in the literature on the effects caused and its magnitude in response to this type of training that uses consecutive short sessions with recovery periods. Thus, the objective of this study was to evaluate the effects of short-term HIIT on markers of oxidative stress and muscle damage in rats.

METHODS

Animals and experimental design

Sixteen male Wistar rats (*Rattus norvegicus*) with an initial age of 60 days were used and maintained under ambient temperature conditions (24 ± 2ºC) and a 12-h light-dark cycle, with free access to filtered water and a standard ration (Labina, Purina®). The animals were randomly allocated into two groups: a control group (n = 8) and a HIIT group (n = 8) and kept in collective cages with groups of four rodents. All procedures were approved by the Ethics Committee for Animal Use at the Institution (15/2017) and were conducted in accordance with the Guidelines of the Brazilian College of Animal Experiments (COBEA).

Procedures

The animals were acclimated and adapted to the liquid environment at a temperature of 25°C ± 1°C in a cylindrical tank 80 cm deep and 80 cm in diameter.[16] In the first week, a 10 min adaptation was performed in the water at a depth of 10 cm. In the subsequent two weeks, the animals had weights applied (contained inside small bags made from cotton cloth and Velcro®) attached to their chests during the adaption period daily for 10 days; the weights used were equivalent to 0%, 1%, and 2% of their body weight, and each animal was subjected to 10 min of swimming exercise with 30 s of swimming and 30 s of rest between the swimming sessions, for a total of 10 sessions. It is important to note that the adaptation period was not capable of inducing possible physiological changes due to the low intensities of exercise used.[17]

The training was performed according to the protocol adapted from Terada et al.,[7] which consisted of 14 swimming sessions lasting 20 seconds with 10 second rest intervals between each session, performed daily for 12 consecutive days, with a load of 14% of body weight and a 60 cm depth of water. After each training session all animals were thoroughly dried to avoid physiological complications from cold and humidity. In an earlier study[10] have identified that the use of this load is considered high intensity for rats.

Twenty-four hours after the end of the last exercise period, the animals were anesthetized with ketamine/xylazine (75 mg/kg + 10 mg/kg ip) and blood (± 5 mL) was collected through cardiac puncture. Then they were euthanized by desalination under anesthesia. After collected the blood it was immediately centrifuged at 800g for 15 minutes at 4°C. The supernatant was then stored in an Eppendorf at –80°C for further analysis of markers of muscle tissue damage. Liver and gastrocnemius muscle tissues were removed and then washed 3 times with a 1.15% KCl solution (Vetec, LTDA, Rio de Janeiro, Brazil), dried and weighed for further analysis of the oxidative stress markers.

For measurement of lipid peroxidation products by means of a thiolarbituric acid reactive substances test (TBARS), the method described by Bose et al.[18] was used.

To determine the oxidative damage caused in the tissues, the products of lipoperoxidation were measured by the oxidation technique of xylenol orange, in which the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) occurs under acidic conditions, catalyzed by lipid hydroperoxides (HPx).[19] The protein concentration of the hepatic and muscular tissues was determined in triplicate by the method of Lowry.[20]

For the determination of superoxide dismutase (SOD) activity, the method described by Madesh and Balasubramanian[21] was used. In the determination of catalase (CAT), the protocol used was that of Nelson and Kiesow.[22] The activity of the enzyme was expressed by the difference in absorbance variation (ΔE)/minute/milligram of protein.[22]

The activity of the enzyme glutathione peroxidase (GPx) was determined according to the method described by Paglia and Valentine.[23] The activity of GR was determined according to the method of Carlberg and Mannervik.[24] For the total sulfhydryl (SH), the method described by Sedlak and Lindsay[25] was used.

The quantification of tissue damage caused by HIIT was assessed by measuring enzymatic markers of tissue damage, such as creatinine kinase (CK) and lactate dehydrogenase (LDH). For quantification, a commercial kit (Labtest®, Lagoa Santa, Minas Gerais, Brazil) was used. Serum (20 µL) from each animal was homogenized in the specific reagents at 37±0.2°C, and the readings were performed using a spectrophotometer (BioSpectro Model SP-22 UV/Visible, Minas Gerais, Brazil) at a wavelength of 340 nm.

Data were expressed as mean ± standard deviation. The normality of the data was tested by the Shapiro-Wilk test. To evaluate the significance of the differences between the means, Student's t-test was used for non-paired samples. Values were considered statistically significant when $p < 0.05$. For all of these procedures, the statistical program GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA) was used.

RESULTS

The evaluations of markers of oxidative damage in hepatic and muscular tissues are shown in Table 1. MDA had a 17.75% reduction in the HIIT group in comparison to the control group in the liver ($p = 0.0482$); however, there were no significant differences in the levels of the hydroperoxides (HPx) or carbonylated proteins (CP). In the gastrocnemius muscle, no significant differences were found in any of the markers evaluated.

For antioxidant activity, there was a reduction (−31.80%) of SOD in hepatic tissue in animals subjected to HIIT when compared with the control group ($p = 0.0375$). However, there were no differences in CAT, GPx, or GR, or the non-enzymatic activity of total sulfhydryl (SH). There were no significant changes in the antioxidant activity of SOD, CAT, GPx, GR or SH in muscle tissue. (Table 2) In the evaluation of the markers of muscle damage (CK and LDH) they were similar between the groups, without significant alterations. (Table 3)

DISCUSSION

According to the results of the present study, short-term HIIT did not promote oxidative or muscular damage. Although a number of biochemical and tissue injury markers were evaluated, no significant changes were observed in most of these markers after physical training when compared with the control group.

Table 1. Effects of HIIT on oxidative stress markers.

	CG (n = 08)	HIIT (n=08)	p (value)
MDA			
Liver	10,81 ± 0,8178	8,891 ± 0,3431*	0,0482
Muscle	7,496 ± 0,5215	6,893 ± 0,4350	0,3922
CP			
Liver	364,7 ± 23,11	317,5 ± 16,71	0,1197
Muscle	235,2 ± 13,90	233,4 ± 11,81	0,9212
HPx			
Liver	3,313 ± 0,3340	2,850 ± 0,2514	0,2873
Muscle	3,113 ± 0,1968	3,488 ± 0,2083	0,2117

Data are presented as mean and standard deviation. Student t test. * Significant difference within the group ($p < 0.05$). malondialdehyde (MDA), carbonylated proteins (CP) and hydroperoxides (HPx).

Table 2. Effects of HIIT on antioxidant activity.

	CG (n = 08)	HIIT (n=08)	p (value)
SOD			
Liver	0,05188 ± 0,005426	0,03538 ± 0,004702*	0,0375
Muscle	0,0355 ± 0,003322	0,03725 ± 0,004078	0,7443
CAT			
Liver	0,01571 ± 0,002514	0,0155 ± 0,002383	0,9517
Muscle	0,0215 ± 0,005207	0,01013 ± 0,002489	0,0534
GPx			
Liver	0,2825 ± 0,01906	0,3543 ± 0,04309	0,1347
Muscle	3,829 ± 1,638	1,18 ± 0,4813	0,1684
GR			
Liver	0,1238 ± 0,017	0,1171 ± 0,01629	0,7851
Muscle	0,2138 ± 0,02732	0,2475 ± 0,01849	0,3236
SH			
Liver	364,7 ± 23,11	317,5 ± 16,71	0,1197
Muscle	235,2 ± 13,90	233,4 ± 11,81	0,9212

Data are presented as mean and standard deviation. Student t test. * Significant difference within the group ($p < 0.05$). superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and sulfhydryl (SH).

Table 3. Effects of HIIT on markers of muscle damage in plasma.

	Control group (n = 08)	HIIT (n=08)	p (value)
CK	170 ± 35,28	194,3 ± 57,98	0,7757
LDH	64,76 ± 36,5	99,17 ± 22,5	0,4346

Data are presented as mean and standard deviation. Student t test. creatine kinase (CK) and lactate dehydrogenase (LDH).

Physical exercise improves the capacity of the cellular antioxidant defense systems in the sense of a neutralization of increases of EROs, and in addition, it improves the metabolic state and insulin sensitivity.[2] However, the intensity of exercise required to produce the most favorable changes in redox homeostasis, the balance between ROS and antioxidants, has not yet been established.[3]

One of the indicators of oxidative stress is the quantification of the products of lipid peroxidation through the TBARS method, which consists of the analysis of the end products as lipid peroxides, malondialdehydes, and other low molecular weight aldehydes.[5] In the present study there was a lower production of malondialdehydes (MDA), corroborating the findings of Bogdanis et al.[13] who confirmed this information in humans after only nine sessions with an approximate duration of 2 to 3 minutes of exercise in each session. However, in the study by Casuso et al.[14] for the marker HPx, they did not find any significant changes in elite swimmers.

In our results we found reductions in TBARS and SOD in the liver, contrary to the findings of Songstad et al.,[15] who also investigated the effects of HIIT conducted over 15 sessions in rat hepatic tissue using treadmill running, and did not identify any significant differences in their proposed animal model.

A decrease in total capacity and an increase in MDA level, together with an increase in SOD activity, are indicators of oxidative stress.[13] Afolayan et al.[26] demonstrated that when ATP concentrations are decreased and ADP concentrations increased, SOD does not dissociate by reducing its mitochondrial concentration. Our finding of a reduction in SOD after the HIIT protocol was an unexpected response and this mechanism needs to be better clarified; however, this may have occurred because in this model of HIIT in swimming, hypoxia may have occurred and limitations in oxygen availability may reduce antioxidant capacity.[2] Corroborating the results found, Casuso et al.[14] in only 6 sessions of HIIT using a swimming model in athletes also found a significant decrease in SOD relative to normal conditions.

In the present study, there was no increase in CP, suggesting that in the HIIT group a protective effect may have occurred, and in other exercise models such as soccer, plyometrics have induced an increase in them and in TBARS.[27] In addition, in the study by Mallard et al.[28] who investigated the effects of HIIT during 36 sessions in men and women, they also did not find a significant difference, results that corroborate the results presented in this study even though a much smaller number of sessions were performed.

EROS can also affect several cellular targets, such as: DNA, proteins, membrane phospholipids, and polyunsaturated fatty acids, favoring the extravasation into the plasma of intracellular enzymes such as CK and LDH.[3] CK is an important indirect marker of muscle damage[13] and in the present study no significant changes were observed in this marker.

Consistent with the present study, Horii et al.,[29] who used the same protocol in 24 sessions and with a load of 16% of body weight, did not find significant differences in the LDH marker.

De Araújo et al.[10] also did not find significant differences in the CK and LDH markers, results very similar to the findings of this study; however, they used a jumping exercise in liquid medium and with a body weight load attached to the animal's back.

Powers and Jackson[5] reported that lipid peroxidation, oxidation, and protein inflammation can promote muscle cell damage, and these mechanisms can affect structural and contractile proteins. On the other hand, the antioxidant defense system may play an important role by attenuating oxidative changes or promoting a faster recovery in response to intense effort, with consequent improvements in performance.[6]

Powers and Jackson[5] pointed out that the CAT enzyme reduces hydrogen peroxide in water, thereby preventing the production of the hydroxyl radical, which can be extremely harmful to tissues. In the present study, there was no statistical difference between the CT and HIIT groups in

CAT. However, CAT expression in anaerobic exercise is controversial[7]. In the study by Casuso et al.,[14] no significant differences were found either.

In the study by Mallard et al.[28] performed in humans, no significant differences were found in the GPx enzyme, further corroborating Songstad et al.,[15] who investigated the same enzyme in animals and also did not find any significant differences; however, the model of exercise performed was of 15 sessions of running on a treadmill.

Maintenance of the antioxidant repair capacity is a response to oxidative stress,[5] however, there was no increase in the antioxidant defense in the present study, since there were no significant alterations in the hepatic and muscular tissues of SH, CAT, GPX, or GR in the HIIT group. It is important to note that sulfhydryl groups are structures associated with proteins and are susceptible to oxidative damage, and thus can be used for the quantification and identification of tissue damage. However, a high level of training increases the skeletal muscle's ability to rapidly detoxify the ROS produced in the exercised muscle.[5]

De Araújo et al.[10] also found no significant differences in SH, SOD, or CAT enzymes in Wistar rats that underwent a HIIT model with jumps in water over 36 or 72 sessions. Pimenta et al.,[12] using a HIIT protocol similar to the present study, but with a gradual increase of the load in relation to the percentage of body weight over 24 sessions, also did not find significant differences in the antioxidant enzymes SOD, CAT, GPx, and GR. In another treadmill running study, Bowen et al.[30] in 16 sessions also found no significant changes in SOD, CAT, or GPx.

CONCLUSION

According to the results presented in this study we can conclude that short-term HIIT did not cause oxidative stress or muscle damage in rats subjected to twelve consecutive sessions.

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

AUTHORS' CONTRIBUTIONS: Each author made significant individual contributions to this manuscript. LMVS was the lead researcher, responsible for the concept/design, data collection, data analysis/interpretation and drafting the article. DGM, ACM, SLS, RAC participated in the concept/design, data analysis/interpretation and drafting of the article. FJA, RMS and JLS performed statistical analysis and participated in the data interpretation, drafting and critical review of the article. LMVS and ANS participated in the data analysis/interpretation, drafting and critical revision of the article. FJA, BGATC, CSE, SSA contributed to concept/design and participated in the data analysis/interpretation and critical revision of the article. All authors read and approved the final manuscript.

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