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original article

Effect of different frequencies weekly training on parameters of oxidative stress

Efeito de diferentes frequências semanais de treinamento sobre parâmetros de estresse oxidativo

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Abstract – Intense muscle contraction induced by physical exercise increases the production of reactive oxygen species, which causes oxidative stress in several organs, such as the liver and the heart. Physical training may increase antioxidative defenses and decrease oxidative stress. However, it is not clear what training frequency improves oxidative stress parameters. This study evaluated the effect of training two and three times a week on oxidative stress biomarkers in the liver and the heart. Eighteen young male mice (CF1) weighing 30 to 35 g were divided into three groups (n=6): no training (NT); twice a week training (T2); and three times a week training (T3). The training program lasted eight weeks, and the animals were killed 48 hours after the last training session. The liver and the heart were removed and stored at -70o C. The following analyses were conducted: thiobarbituric acid reactive substances, protein carbonylation, total thiol content, superoxide dismutase, catalase and glutathione peroxidase. Oxidative damage was reduced only in the T3 group, and there was an increase in total thiol content, supeoxidase dismutase and catalase in T3 when compared with the NT group. Glutathione peroxidase was not significantly different between groups. Only training three times a week seemed to reduce oxidative stress and increase the efficiency of the antioxidant system in mice.

Key words: Antioxidant enzymes; Oxidative damage; Physical exercise; Training frequency.

Resumo – *Durante a contração muscular intensa induzida pelo exercício físico, há aumento na produção de espécies reativas de oxigênio, ocasionando estresse oxidativo em diversos órgãos, dentre eles o fígado e o coração. O treinamento físico pode aumentar as defesas antioxidantes e diminuir o estresse oxidativo. Contudo, ainda existem dúvidas sobre a frequência de treinamento necessária para melhorar parâmetros de estresse oxidativo. Este trabalho tem como objetivo verificar o efeito das frequências de duas e três vezes de exercício por semana sobre biomarcadores de estresse oxidativo no fígado e coração. Foram utilizados 18 camundongos machos (CF1), jovens (30 a 35g) divididos em grupos (n=6/grupo): não treinado (NT); treinado duas vezes por semana (T2) e treinado três vezes por semana (T3). Os animais foram submetidos ao treinamento durante oito semanas. Quarenta e oito horas após a última sessão os animais foram sacrificados. O fígado e o coração foram removidos e armazenados em freezer – 70ºC. Foram analisadas as substâncias reativas ao ácido tiobarbitúrico, carbonilação de proteínas, conteúdo total de tióis, atividades da superóxido dismutase , catalase e glutationa peroxidase. Os resultados demonstraram que apenas o grupo T3 reduziu dano oxidativo. Ademais, houve aumento no conteúdo total de tióis, atividades da superóxido dismutase e catalase no mesmo grupo em comparação com o não treinado. A atividade da glutationa peroxidase não apresentou diferença significativa entre os grupos. Este estudo demonstrou que somente a frequência de treinamento de três vezes por semana reduz dano oxidativo e aumenta a eficiência do sistema enzimático antioxidante de camundongos.*

Palavras-chave: *Antioxidantes; Estresse oxidativo; Exercício físico.*

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INTRODUCTION

During muscle contraction induced by exercise, oxygen consumption may increase 10 to 20 times in the system and 100 to 200 times in muscles when compared with resting values[1]. This increase may cause a concomitant elevation in the production of reactive oxygen species (ROS)[2,3].

The unbalance between ROS production and removal leads to oxidative stress (OS), which is associated with muscle damage and metabolic disorders and, consequently, a reduction in physical performance[2,4]. In addition, OS affects the physiological functioning of several organs, such as the liver and the heart. The high metabolic rate of these organs is associated with the high flow of electrons in the mitochondrial respiratory chain and the consequent high production of ROS[5].

The liver is the main organ in metabolic control, and several authors suggest that it is substantially affected by OS during and after physical exercise[6,7]. In the same way, the heart, the central organ of the circulatory system, also has a high oxygen consumption during exercises, which favors the production of ROS. ROS are produced primarily due to an electron leak at the level of the coenzyme Q between complexes I and III of the electron transport chain (ETC). Moreover, xanthine oxidase found in cytosol and the membrane-bound NADPH oxidase are important sources of ROS production during physical exercise[1]. Oxidative stress leads to several changes in liver and heart cells and may cause diseases such as liver steatosis, hepatitis C and atherosclerosis. However, physical training, when carefully planned, may improve both antioxidative defense mechanisms[5,6,8] and the oxidative capacity of tissues,[9] which may decrease the magnitude of the oxidative attack[10,11] and prevent its deleterious effects[12,13].

Previous studies with animal models have confirmed that physical training positively affects the redox state of innumerable cells and tissues and may improve oxidative stress parameters[5,10,14,15]. Those findings might be applicable to human beings as there is a link between the different animal species and data found in animal studies may be extrapolated to the human species. Silva et al.[5] and Frederico et al.[12] demonstrated that eight weeks of training with five weekly sessions was enough to reduce oxidative stress in the liver and the heart[5,12]. However, it is not known whether frequencies of two and three weekly session during eight weeks of training are sufficient to also induce improvements in OS parameters in liver and heart tissues. This study evaluated the effects of training two and three times a week during eight weeks on oxidative stress parameters in the liver and heart of mice.

METHODS

This study, conducted in accordance with the Guide to the Care and Use of Experimental Animals[16], was analyzed and approved by the Ethics Committee of Universidade do Extremo Sul Catarinense, Santa Catarina, Brazil. Eighteen male mice (CF1) aged about 90 days and weighing 30 to 35 g were

obtained from the laboratory animal facility of Universidade do Extremo Sul Catarinense. The animals were housed in collective polypropylene cages and fed water ad libitum and a balanced chow (Purina®). During the experiment, all animals were kept in a room at a controlled temperature of about 23° C and a 12-h light:dark cycle.

STUDY PROTOCOL

The mice were randomly divided into three groups (n=6): no training (NT); twice a week training (T2); and three times a week training (T3). All animals underwent adaptation in an ergometric treadmill for one week (10 m/min, no incline, 10 min/day) everyday of the week. After adaptation, animals in T2 and T3 groups received eight weeks of training (treadmill running) at a constant speed of 13 m/min, no incline, and 45 minutes per session[9]. This training speed corresponds to a moderate intensity of 78% of VO_2max[17]. Forty-eight hours after the last training session, the animals were anesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (12 mg/kg) and then killed. The liver and the heart were surgically removed and immediately stored in a freezer at -70° C for later analysis.

Oxidative damage markers

Oxidative lipid damage was determined according to the formation of reactive substances when heating thiobarbituric acid (TBARS), which was measured spectrophotometrically (532 nm) and expressed in nmol/mg/protein, as described by Draper & Hadley[18].

Oxidative damage in proteins was measured according to carbonyl groups based on the reaction with dinitrophenylhydrazine. Carbonyl content was determined spectrophotometrically (370 nm) using a coefficient of 22,000 molar-1 in mmol/mg/protein, as described by Levine et al.[19].

Total thiol (TT) content was determined using a reaction of two thiol groups with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), which generated a yellowish derivate. TT content was read spectrophotometrically (412 nm) and expressed in DTNB/mg/protein[20].

Antioxidant enzyme activity

The enzyme activity of superoxide dismutase (SOD) was determined by inhibition of auto-oxidation of adrenalin measured spectrophotometrically (480 nm) and expressed in U of SOD/mg/protein, as described by Bannister & Calabrese[21].

Catalase (CAT) activity was determined according to the decrease in the consumption of hydrogen peroxide measured spectrophotometrically (240 nm) and expressed in U of CAT/mg/protein, as described by Aebi[22].

Glutathione peroxidase (GPX) activity was determined according to the rate of NADPH oxidation measured spectrophotometrically (340 nm) and expressed in mM/min/mg/protein, as described by Flohé and Gungler[23]. The amount of protein in all assays was measured using the Lowry et al. technique[24].

Statistical analysis

Data were expressed as mean and standard error of the mean and analyzed statistically using one-way analysis of variance (ANOVA) followed by the Tukey test. The level of significance was set at 5% ($p<0.05$). The software used for data analysis was the Statistical Package for the Social Sciences (SPSS®) 17.0 for Windows.

RESULTS

Figure 1A shows that the level of TBARS in the liver (0.13±0.02 nmol/mg/protein) and in the heart (0.20±0.01 nmol/mg/protein) in the T3 group was lower than in the NT group (0.25±0.02 and 0.36±0.06 nmol/mg/protein). The results of protein carbonylation (Figure 1E) also resulted in a decrease in carbonyl content in the liver (0.19±0.049 nmol/mg/protein) and the heart (0.15±0.011 nmol/mg/protein) in the T3 group when compared with the NT group (0.35±0.041 and 0.26±0.017 nmol/mg/protein). However, training twice a week (T2) did not change TBARS levels or carbonyl contents in the liver (0.23±0.02 and 0.32±0.09) and the heart (0.32±0.03; 0.24±0.04 nmol/mg/protein) when compared with the NT group. The results of total thiol (Figure 1C) showed a greater content in the liver (71.08±4.79 DTNB/mg/protein) and the heart (97.7±14.2 DTNB/mg/protein) in the T3 group than in the NT group (41.7±4.07 and 41.6±8.3 DTNB/mg/protein). However, training twice a week did not affect this marker significantly (47.7±4.2 and 51.9±5.8 DTNB/mg/protein) when compared with the NT group.

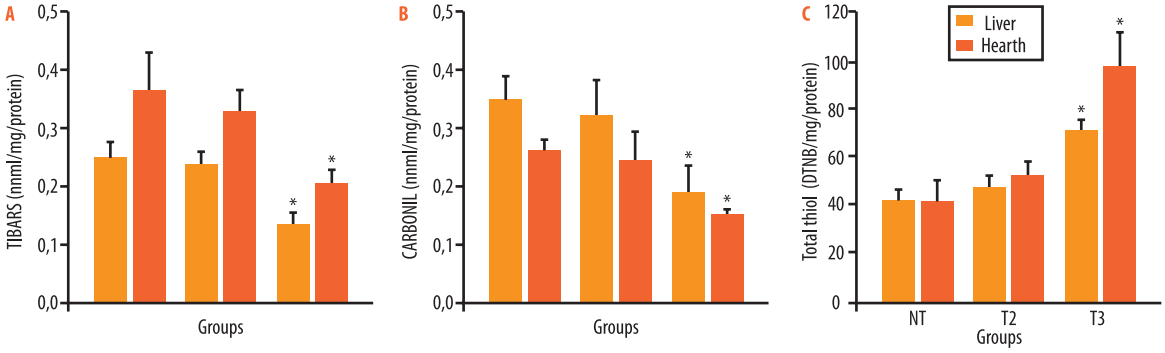

Figure 1. Lipid peroxidation (A), protein carbonylation (B) and total thiol content (C) in the liver and heart of mice 48 h after the last training session. Values were described as mean ± SEM and analyzed using ANOVA and the Tukey test. Lipid peroxidation and protein carbonylation were expressed in nmol/mg/protein, and total thiol content, in DTNB/mg/protein ($p < 0.05$ vs. NT).

The results of the analysis of antioxidant enzymes (Figure 2A) showed an increase of SOD in the liver (0.37±0.05U/mg/protein) and in the heart (0.23±0.02 U/mg/protein) in the T3 group when compared with the NT group (0.15±0.01 and 0.12±0.01 U/mg/protein). However, training twice a week (T2) did not affect this marker significantly (0.24±0.03 and 0.07±0.006 U/mg/protein). The results of the analysis of CAT (Figure 2B) showed an increase in the liver (0.17±0.03 U/mg/protein) and in the heart (0.45±0.04 U/mg/protein) in the T3 group when compared with the NT group (0.07±0.02

and 0.02±0.001 U of CAT/mg/protein). Again, training twice a week (T2) did not significantly increase CAT activity in the liver (0.05±0.01) and in the heart (0.02±0.001 U/mg/protein) when compared with the NT group. The GPX activity (Figure 2C) in groups T2 and T3 in the liver (0.8±0.06 and 1.0±0.1 mM/mg/protein) and in the heart (0.7±0.1; 0.9±0.1 mM/mg/protein) was not significantly different from that in the NT group (0.7±0.1 and 0.6±0.04 mM/mg/protein).

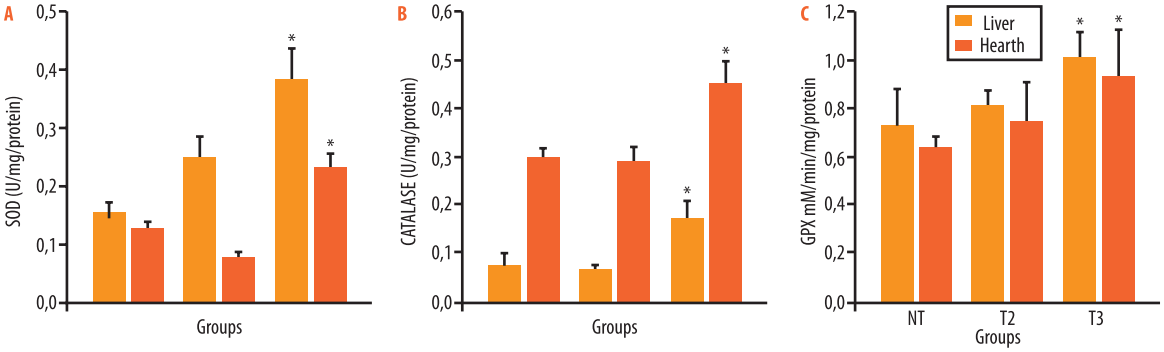

Figure 2. Superoxide dismutase (A), catalase (B) and glutathione peroxidase (C) in the liver and heart of mice 48 h after the last training session. Values were described as mean ± SEM and analyzed using ANOVA and the Tukey test. Superoxide dismutase and catalase were described in U/mg/protein, and glutathione peroxidase, in nM/min/mg/protein ($p < 0.05$ vs. NT).

DISCUSSION

Studies have shown that exhaustive physical exercise increases the production of ROS and, consequently, leads to OS in several organs and tissues[6,7]. However, other studies found that moderate chronic exercise produces metabolic adaptations that may help to reduce OS in several organs, particularly in special populations[13,14].

In their analysis of physiological adaptations as a result of the practice of physical exercise in twice a week exercise sessions, Dalleck et al. [25] found improvements in physiological training parameters (lactate and VO_2max). However, the effects of biochemical responses on oxidative stress markers remain unclear. The results of this study showed that two training sessions per week is not a sufficient frequency to promote improvements in OS parameters. The long interval (>72 h) between sessions may exceed the supercompensation phase and may inhibit the biochemical adaptive effect of training.

The association between OS and physical exercise is directly associated with training intensity and duration[2,26]. Therefore, training should have a sufficient intensity and duration to create an adaptive response of the organism in each session. Our results suggest that exercise sessions at least three times a week for eight weeks are necessary to reduce oxidative damage and to increase the activity of antioxidant enzymes in the liver and in the heart of animals.

ROS attack lipids and cell proteins and steal electrons to achieve a stable chemical state[1]. The results of our study also showed that ani-

mals that had training sessions at least three times a week had lower levels of lipid damage (TBARS) and protein carbonylation (PC). Our findings confirm the results of several studies that found a reduction in oxidative stress parameters after similar training programs (three times a week)[14,23].

The reduction of oxidative damage induced by physical training may be explained by at least three main mechanisms: first, the increase of both the expression[12] and the activity[9] of antioxidant enzymes; second, the reduction of oxidant production[13] and, also, the lower electron leakage from mitochondria[9]; and third, the chronic exposure of tissue to ROS, induced by training, which makes the organ more resistant to the effects that derive from the mechanisms of oxidative stress[10].

ROS may affect amino acids in chain reactions by means of protein aggregates susceptible to proteolysis. During this process, some amino acids are converted into carbonyl derivates[1]. It is clearly established in the literature that oxidized proteins are less degraded by proteasomes. These intracellular proteases are responsible for 70% to 80% of degradation after exposure to oxidants and play an essential role in the antioxidant system[28]. One of the possible mechanisms to reduce the levels of PC, according to Radák et al.[11], is that physical training increases proteasome activity in adaptation to protein oxidation, which accelerates their repair (protein turnover).

Another important marker of protein oxidation is total thiol (TT) content. Our study found an increase in TT only in the T3 group. The technique used measured non-oxidized sulfhydryls in amino acids[20]. The SH group may be oxidized by free radicals, which compromises protein functioning. A possible explanation for these results is the increase of stress proteins (HSP) induced by exercise[26]. The function of these proteins is to control cell homeostasis and to protect against excessive oxidation. However, HSP measurement was not carried out here, which is a limitation of our study.

The analysis of antioxidant enzymes showed that there was an increase in SOD and CAT only in the groups that received training three times a week. SOD converts superoxide radicals ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2), which is, subsequently, catalyzed by CAT and converted into water and molecular oxygen. Some studies have argued that physical training has no effect on antioxidant enzymes in the liver and in the heart[29]. However, the results of our study, in agreement with other findings, showed that physical training increases SOD activity in the liver[5,6] and in the heart[15].

After physical training, SOD activity increases, probably in response to oxidative stress induced by exercise. This finding may be explained by the fact that regular physical training activated transcription factors, such as NF-kB, responsible for activating a variety of genes, and mitochondrial SOD[30].

The effect of training on CAT activity and expression is unclear and controversial[10]. However, CAT activity is higher in the liver and heart of

trained rats[8]. Physical training activates transcription factors, such as AMPK, which activate CATmRNA and stimulate its protein synthesis, which may increase its activity[1,8]. Moreover, the high activity of CAT may be assigned to H_2O_2 formation by SOD. According to Halliwel and Gutteridge[1], the chemical interaction of H_2O_2 in the active site of catalase transfers a hydrogen atom from the first to the second oxygen atom, which leads to heterolytic fission between atoms and forms water (non-deleterious molecule). This, in turn, explains the decrease of oxidative damage in tissues.

In contrast, the activity of glutathione peroxidase (GPX) was not significantly different between the experimental groups in this study. GPX and CAT have similar functions in H_2O_2 decomposition. However, GPX is more efficient when ROS concentrations are high, whereas CAT plays an important role when H_2O_2 is low[3]. Physical training three times a week may promote an adaptive effect in the redox balance of antioxidants so that the low H_2O_2 concentrations are affected only by CAT, a hypothesis that may explain the results described above.

CONCLUSION

The results of this study showed that treadmill training three times a week reduced oxidative damage and increased the efficiency of the enzyme antioxidant system in the liver and heart of mice.

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